# *Sterculia foetida* - Eco-friendly, cost effective and rich sources of nutritious edible oil, animal food supplements as well as biofuel and to evaluate its antimicrobial and cytotoxic efficacy by comparing with other edible vegetable oils to validate its pharmaceutical application


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**2022**

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## To whom it may concern

This is to certify that the thesis entitled "*Sterculia foetida*, L - Eco-friendly, cost effective and rich sources of nutritious edible oil, animal food supplements as well as biofuel and to evaluate its antimicrobial and cytotoxic efficacy by comparing with other edible vegetable oils to validate its pharmaceutical application" submitted by Mr. Rahul Bose who got his name registered on 19.01.2018 for the award of Ph. D. (Science) degree of Jadavpur University, is absolutely based upon his own work under the supervision of Dr. Suparna Mandal Biswas and that neither this thesis nor any part of it has been submitted for either any degree/diploma or any other academic award anywhere before.

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# *Acknowledgement*

*It gives me great pleasure and privilege to express my deep sense of appreciation to my reverend mentor Dr. Suparna Mandal Biswas for her excellent and valuable guidance with endless encouragement, intelligent freedom and stupendous co-operation, care and moral support, which were the most important factors for the success of this work and also taught me how to think like a scientific researcher, especially in the field of phytochemicals.*

*I am deeply indebted to Prof. Panchanan Pramanik for his able guidance, suggestions, constant source of inspiration and endless help throughout the period of my research work.*

*I am very much thankful to Head of the Department, Agriculture and Ecological Research Unit, Prof. Pabitra Banik for allowing me to perform and providing needful facilities throughout the research work.*

*I am also very much thankful to Dr. Raghunath Chatterjee Prof. in. Charge, Biological Sciences Division, Indian Statistical Institute for allowing me to perform my thesis and getting all the important help to fulfil my research work.*

*I offer my gratitude and appreciation to Dr. Arindam Pramanik, School of Medicine, St James's University Hospital, University of Leeds for helping me perform the cytotoxicity detection analysis.*

*I am thankful to Dr. Ramdhan Majhi, Principal Technical Officer, IICB, Kolkata Central Instrument Facility, for providing all the work and data related to fatty acids.*

*I am thankful to Dr. Supriya Majumdar, AERU. ISI Kolkata for helping me to analysis and providing important suggestions regarding the testing of the heavy metals and alkaline earth metals by using the Atomic Emission Spectroscopy.*

*I am also thankful to my junior Mr. Ayan Paul for helping me to perform the statistical analysis of my test samples.*

*I also owe my deepest respect to our Director, Prof. Sanghamitra Bandhopadhyay for providing laboratory facilities and financial support for performing this research work.*

*Success of this research work is also largely due to the co-operation and help which I have received from my seniors and labmates starting from Dr, Nirjhar Dasgupta, Dr. Anjan Hazra, Dr. Debabarata Das and Dr. Supriya Majumdar.*

*My cordial thanks are extended to my friends and colleagues, Miss Ekta Bhattacharya, Miss Rajashree Dutta, Miss Madhurima Dutta, Miss Moumita Ghosh, Miss Anisha Dutta, Miss Sreemoyee Mitra and Mr. Abhishek Bhattacharya for their friendly co-operation and consistent suggestion for completion of my work.*

*Without the love, admiration, encouragement and moral support of my parents Mr. Siddhartha Bose and Mrs. Sanchita Bose and my Late Grandfather Mr. Prabhat Kumar Bose and my Late Grandmother Mrs. Chaya Bose this work would have been impossible to finish. I take this opportunity to thank them all for helping me in shouldering all my burdens during my absence.*

*I also thank Lord Ganesha for helping me whenever I have faced any critical problem and situation and always helped me to overcome that.*

# Abstract

**Title : "*Sterculia foetida* - Eco-friendly, cost effective and rich sources of nutritious edible oil, animal food supplements as well as biofuel and to evaluate its antimicrobial and cytotoxic efficacy by comparing with other edible vegetable oils to validate its pharmaceutical application".**

**Submitted by-Rahul Bose**

**Index No.-56/18/Life Sc./25**

Medicinal plants have long been used in the prevention and cure of various diseases of the humans and the animals. *Sterculia foetida* seed oil consists of the two unusual fatty acids namely sterculic acid, malvalic acids with cyclopropane functionality. Seeds of *Sterculia foetida*, L. yielded considerable amount of oil (58.7g/100g) as compared to other edible vegetable oils (such as sunflower, ground nut, mustard, soybean). Fatty acids composition of all the five tested oils showed that total fatty acid as well as unsaturated fatty acid percentage is higher in Sterculia seed oil. Proximate and mineral composition analysis suggested that Sterculia oil is a good source of protein, lipids, macro and micronutrients. Lowest TOTOX value (2.67) and higher iodine value (132-144) indicated its higher oxidative stability and presence of greater number of unsaturated bonds in the fatty acid moieties which is also beneficial for human health. Sterculia oil exhibited lower $IC_{50}$ values in DPPH (825.73 μg/ml), ABTS (225.23 μg/ml) and NO (111.98 μg/ml) radical scavenging assays, only sunflower and mustard oils showed significant differences in $IC_{50}$ values in DPPH assay. Sterculia oil did not exhibit any cytotoxic effect on both normal and cancerous cell lines even at concentrations of 40μg/ml as evident from MTT assay. Antimicrobial, cytotoxic and molecular docking study (with Bax and MDM2) of bromosterculic acid [8-(1, 2-dibromo-2-octylcyclopropyl)octanoic acid], a new synthetic derivative of *Sterculia foetida* seed oil was assessed to validate its pharmaceutical potentiality. Bromo-sterculic acid was prepared by bromination of sterculic acid and its structure was confirmed by Mass spectrometry. This synthetic derivative showed strong fungicidal activity against two pathogenic fungal species namely *Penicillium chrysogenum* and *Aspergillus niger* with minimum inhibitory concentration (MIC) of 0.007 mg/mL and strong bactericidal activity against *Bacillus subtilis* and *Xanthomonas* sp.with MIC of 0.015 mg/mL. Cytotoxic activity on both normal (MCF-10A) and cancerous (MDA-MB-468) cell lines revealed that survivability rate of normal cells were unaffected, whereas cancerous cells were decreased greatly by bromo-sterculic acid at doses less than 5μg/mL. Molecular docking using Auto Dock 4.2 showed that bromosterculic acid binds to Bax with the best conformation that has a minimum free binding energy of -11.4kcal/mole. It makes strong pi-sigma interaction with PHE-93, pi-alkyl and alkyl interaction with TRP-139, ARG-89 and PHE-92. The best conformation of bromosterculic acid that binds to MDM2 has −11.6 kcal/mol binding energy. It makes strong hydrogen bond interaction with GLN-59 and pi-alkyl interaction with PHE-55.

Signature of the supervisor Signature of the candidate

# Keywords-

i. Edible vegetable oil

ii. *Sterculia foetida* L

iii. Fatty acids

iv. Oxidative stability

v. Cytotoxic activity

vi. Antimicrobial activity

vii. Molecular docking,

viii. Bax, MDM2

ix. P53 inhibitor

x. Oilseeds

# Abbreviations-

i. Mha- Million hectares

ii. Mt- Million tonne

iii. ICMR- Indian Council of Medical Research

iv. GDP- Gross domestic product.

v. WTO- World Trade Organisation.

vi. ICAR- Indian Council of Agricultural Research.

vii. FAME- Fatty Acid Methyl Esterification.

viii. GC-MS- Gas Chromatography and Mass Spectrophotometry

ix. MTT- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

x. CHD- Coronary heart disease.

xi. SFA- Saturated Fatty Acids.

xii. MUFA- monounsaturated fatty acids.

xiii. PUFA- Polyunsaturated Fatty Acids.

xiv. LDL- Low Density Lipoprotein.

xv. TFA- Trans fatty acids.

xvi. RCT- Randomized controlled trial.

xvii. Solvent extracted oil (SEO).

xviii. BAP- N6-benzylaminopurine.

xix. KOH- Potassium hydroxide

xx. HCL- Hydrochloric acid

xxi. FFAs- Free fatty acids

xxii. LC-MS- Liquid chromatography and Mass Spectra

xxiii. MS Q-TOF- Mass Spectrometer Quadrupole Time of Flight.

xxiv. AES- Atomic Emission Spectroscopy.

xxv. SEs- Sterol Esters.

xxvi. DPPH- 2,2-diphenyl-1-picryl-hydrazyl-hydrate.

xxvii. ABTS- 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid.

xxviii. NO- nitric oxide.

xxix. PV- Peroxide value.

# PREFACE:

Medicinal plants have long been used in the prevention and cure of various diseases of the humans and also of the animals. This had been served from the ancient times and had provided a good remedy for the benefit of mankind. Plant derived medicines have thoroughly contributed for the development of human health throughout history, and it is expected that it will continue to prosper playing a vital role in global health systems. From the beginning of the twentieth century, most of the drugs nearly 80% were derived from the plants, but with the advent of organic chemistry and breakthrough research, the paradigm was shifted favouring synthetic drugs. However this trend of using synthetic drugs over natural remedy slowed down silently in the last two three decades with the growing knowledge of using natural products, to find solution of complex medical problems. Then revival in herbal drug usage have become an important trend in both the developed and developing countries. The most important finding of this study can be attributed using sterculic acid as antimicrobial and anticancer agent as natural products from *Sterculia foetida* plants that represent a fertile ground for the development of novel anticancer agents that can be used for the formation of future medicines and also for the treatment of infectious diseases with targeted drug delivery for pharmaceutical industry. Selective drug targeting is the need of the current therapeutic regimens for increased activity on cancer cells and reduced toxicity to normal cells.

According to the World Health Organisation (WHO) it has been estimated that about 80% of the world's population generally depends on traditional medicines for the treatment of various health disorders. Along with medicinal values biodiesel/biofuel concerns in the light of current energy security and environmental pollution has a high potential factor as a solution for renewable energy. Ever decreasing supply of fossil fuel reserves and to make environment more cleaner, renewable energy is an attractive alternative energy source for the future. Biofuel can be a good alternative fuel for diesel/ gasoline powered vehicles and can be produced by the chemical reaction of a fat/oil with an alcohol in the presence of a catalyst. This type of alternate fuel has a lot of technical advantages over fossil fuels such as lower exhaust emission, less toxic gases, biodegradability derivation from a renewable and domestic feed stock, less sulphur content in the air, superior flash point and eco-friendly fuel. The choice of feedstock for present commercial biodiesel producing plants generally depend on the availability of that particular crop. There is an increasing importance to search for suitable alternative oils for the production of biodiesel, as the use of edible oils are being restricted globally and though several nonedible

oils like Jatropha and Karanja are being utilized for biodiesel preparation, but very few unusual fatty acids containing oils such as castor oil and lesquerella have been utilized for this purpose. The hydrocarbon chain length in biodiesel generally varies in the range of 16-20 carbons with and without unsaturation containing oxygen at one end.

*Sterculia foetida* oil contains the two unusual fatty acids namely sterculic acid, malvalic acids with cyclopropane functionality. The oil of sterculia being a tree-borne oilseed based oil has a good scope to utilize this oil as a potential feed stock for biodiesel production. This plant belongs to the *Sterculiacea* family that comprises of about 2000 types of species. It is a wild plant with a common name as 'Java-Olive', 'Bastard poon' tree, 'Hazel Sterculia'. In India it is also known as 'Janglibadam'.It is a large tree that grows upto 4 meters in height and 3 meters in girth with branches arranged in whorls and spreads horizontally. The seeds are exalbuminous type that has a starchy cotyledons and is straight with small radical. It is numerous 3-4 inch long, ellipsoid, oblong, 1.5-1.8 cm slate colored with yellow caruncle on one side at the point. Sterculia plant species are rich in alkaloids, saponins and flavonoids glycosides that showed a wide array of biological activities such as antimicrobial, antifungal, insecticidal , cytotoxic, antioxidant and anti-inflammatory activities. Apart from the pharmacological activities of the secondary metabolites there has been a myriad of pharmaceutical application of its natural gums derived from its tree exudates such as gum Karaya (*Sterculia urens*) and chicha gum (*Sterculia striata*). They are used as stabilizers, disintegrators and also active ingredient release enhancers.

Thus oil of Sterculia has all the properties of both to be used as a medicinal plant having good antimicrobial activity compared to other oils present in this experiment, but also to use it as a good source of biofuel. The oil content from the seeds are much higher compared to other oilseeds, and it consists of all the essential nutrients (alkaline earth metals, heavy metals), proteins, lipids and also shows lower totox value and higher iodine value indicates its higher oxidative stability. Sterculia oil exhibited lower $IC_{50}$ values in DPPH, ABTS and NO radical scavenging assays, only sunflower and mustard oils showed significant differences in $IC_{50}$ values in DPPH assay. Sterculia oil did not exhibit any cytotoxic effect on both normal and cancerous cell lines as evident from MTT assay. Bromo-sterculic acid [8-(1, 2-dibromo-2-octylcyclopropyl) octanoic acid] was prepared by bromination of sterculic acid and its structure was confirmed by Mass spectrophotometer analysis. This synthetic derivative showed strong fungicidal activity against two pathogenic fungal species namely *Penicillium chrysogenum* and

*Aspergillus niger* with minimum inhibitory concentration (MIC) value of 0.007 mg/mL and good bactericidal activity against *Bacillus subtilis* and *Xanthomonas sp.* with MIC value of 0.015 mg/mL. Cytotoxic activity on both normal and cancerous cell lines revealed that survivability rate of normal cells were unaffected, whereas cancerous cells were decreased greatly by bromo-sterculic acid with 50% survivability. Molecular docking using AutoDock 4.2 with Bax exhibited strong pi-sigma interaction with PHE-93, pi-alkyl and alkyl interaction with TRP-139, ARG-89 and PHE-92 whereas MDM2 revealed strong hydrogen bond interaction with GLN-59 and pi-alkyl interaction with PHE-55.Based on all the parameters it can be concluded that this oil can be used as a good alternative and viable source of safe edible oil and also as biofuel to meet the future energy demands and to keep the environment and earth clean.

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## Introduction:

Vegetable oils are an important part in the production of manufactured foods both in the domestic and international markets (**Baker,P. and Friel, S.,2014**).The demand for the vegetable oil market is increasing at a rapid rate of 3.25% during the forecast period (2019-24)( **Popescu *et al*., 2019**). Due to increase in demand in the last 30 years oil crop production increased by at least 240%, while the increase of cultivable land and yield increases by about 82% and 48% respectively. Soybean is the main oilseed produced other than remaining oilseeds where it represents more than 50% of the total oilseeds produced in the world **(Sharma *et al*., 2012).** Oilseeds occupy an important position in the agricultural economy of India and being the largest producer of oilseeds in the world contributes nearly about 7% of the global vegetable oil production with about 14% of the land area (**Jha *et al*., 2012**). Considering about 19% of global area with around 2.7% of global production, these oilseed crops hold the second most important determinant of Indian agricultural economy only next to cereals **(Reddy, V.K. and Immanuelraj, K.T., 2017)**. With under 27 Mha area under oilseeds cultivation with an average yield of 1095 kg/ha yielding 29 Mt, India conquers a significant position in the world as fourth leading oilseeds producing countries lacking behind only to USA, China and Brazil. Oilseed crops has attained annual growth rate of 2.44%, 5.47% and 2.96% at area, production and yield respectively during last decade (1999-2009**) (Ghosh *et al*., 2018).**

Oilseeds are the major crops that are grown in India apart from rice, wheat, maize, sorghum and other cereals (**Rai, *et al.,* 2016**). In terms of area, production and economic value these crops are second in production to the food grains available in our country.India has the second largest oilseeds cultivable area in the world next to USA but in case of global economy

regarding vegetable oil it is placed fourth next to USA, China and Brazil. India has annual turnover of about Rs 80000 crore and accounts for about 12-15 percent of oilseeds area,7-8 percent oilseeds production (**Kumar *et al.*, 2005**),6-7 percent of vegetable oil production,9-12 percent of vegetable oil import and 9-10 percent of edible oil consumption **(Edible oil supply and demand scenario in India, Girish Kumar Jha *et al.*, Division of Agriculture economics)**.

With the increase in demand of domestic vegetable oil consumption, the production has not been up to the expectations to keep up pace with the ever rising demands (**Asif, M. and Muneer, T., 2007)**. Low and unstable yields of most oilseed crops and uncertainty in returns to investment results from the continuing cultivation of oilseeds in rain fed high risk production environments. These are the factors leading to the situation of high demand supply. The per capita consumption of fats through vegetable oils in India is nearly 6.4kg compared **(Rahman, A., 2016)** with the minimum nutritional standard prescribed by the ICMR (14kg/capita/annum). The growth rate of oilseeds in India is around 2% per annum which is lower than the annual population growth of around 2.2%. From the total annual consumption of 168 million tonnes India imports nearly half of it (**Jha *et al.*,2012**). The Technology Mission on Oilseeds (TMO) launched in 1986 was the first comprehensive intervention aiming self-sufficiency in edible oils production through the spread of technology and provision of market support **(Gulati *et al.*, 1996).** But rise in demand for edible oils made an unsubstantiated increase in the imports at a large quantities leading to a substantial drain on foreign exchange .Edible oil imports increased from a mere 15 percent of total edible oil consumption in 1995-96 to nearly 53 percent in 2009-2010 **(Narayan, P., 2016).**

India with its rich agro-ecological diversity provides a favourable condition to cultivate all the major nine oilseed crops. Among the nine oilseed crops developed in the country, seven are of edible oils (soybean, groundnut, rapeseed-mustard, sunflower, sesame, safflower and niger)

and two are of non-edible oils (castor and linseed ).India ranks first in the production of most of the minor oilseeds (castor, niger, safflower and sesame) **(Rai *et al*., 2016)**. In case of the major oilseeds, India ranks first in the production of groundnut, second in rapeseed-mustard and fifth in soybean. A major contributor to the agricultural GDP are the oilseed crops. In 2009-10 the area under the nine oilseed crops was nearly 26.11 M ha with production of about 24.88 Mt**,(Sharma, P., 2016)** and the total edible oil production in the country stood at 6.17 Mt. India's oilseed and edible oil sector is always exposed to International markets and it influences the policy options like the minimum support price and other market intervention policies which has not been able to generate the desired changes along with the needs and target, the productivity trends in annual edible oilseeds having shown considerable variability in response to the prevailing policy in environment and priority. **[ CSO (2013), "National Accounts Statistics 2013", Central Statistics Office, Ministry of Statistics & Programme Implementation, Government of India, New Delhi, May 2013**].

Besides the nine major oilseeds grown in India, a number of minor oilseeds of horticultural and forest origin including coconut, palm oil , castor oil and linseed oil are also cultivated (**Thapa *et al*.,2019)**. In addition subsequent quantities of vegetable oil are also obtained from rice bran, cotton seed oil along with small quantity from tobacco seed and corn (**Hegde, D.M., 2012**). According to the census of 2009-2010 the total edible oil production in the country stood at 6.17 metric tonne. The total oilseeds production in the country and the area where it is cultivated in huge amount are located mostly in the central and southern parts of India, mainly in the states of Madhya Pradesh, Gujarat, Rajasthan, Andhra Pradesh and Karnataka **(Krishna Kumar *et al*., 2004**). In case of other oilseeds groundnut, rapeseed-mustard and soybean account for about 80 percent of area and 87 percent of the production of oilseeds in the country (2010-2011).

The domestic demand for edible vegetable oils has been increasing rapidly each year at a rate of 6 percent per annum, but the domestic output has been not enough to meet the demands which is just at a rate of about 2percent per annum (**Srinivasan, P.V., 2012**). Compared to other countries the average yield of most oilseeds are much lower in India. The cultivation of oilseeds in India is in high risk condition where there are uncertainty of the returns on the investments. The area , production and productivity of oilseeds grew at a compound annual growth rate of 1.58%,3.05 % and 1.45 %, respectively, during the period 1951-2009. Among the oilseed crops, the growth rate in area and production was the highest for soybean (10.74 % and 12.73 %, respectively). ( **CSO (2013), "State-wise Estimates of Value of Output from Agriculture and Allied Activities 2013, with New base Year 2004-05 (2004-05 to 2010-11), Central Statistics Office, Ministry of Statistics & Programme Implementation, Government of India, New Delhi, May 2013**.)

As India has not been capable to meet the demand of vegetable oil in the domestic market in abundant, the amount of edible oil to be imported showed an increasing trend (**Panda, H., 010).** The edible oil import increased from 4.9Mt in 2007-2008 to 8.1Mt during 2009-10.

This dependency on import of edible vegetable oil on a regular basis acts as a significant drainage of foreign exchange reserves of the country. The per capita consumption of edible oil is 12.7 kilogram per annum which is well below the world average of 23.46 kg/ annum **(Sharma, P., 2016).** Imports of edible oil in the country slacken during 1996-1997. The policy of liberalization formed its path as much from the lack of self-sufficiency in domestic edible oil production as from the commitments under the new multilateral trade regime under WTO. This result to change in the cropping patterns after liberalization of edible oil imports in 1996-97. Change in the cropping patterns gave rise to the growth of other minor oilseeds of horticultural and forest origin **(Pandey, G. and Kumari, S., 2021)**. This include particularly coconut oil and palm oil also grown in the country. Along with this substantial quantities of

vegetable oil are also been obtained from rice bran and cotton seed along with small quantity from tobacco seed and corn **(V. Muralidharan and N. Manivanan, 2006 ).** The area under oilseed production and the amount of obtained product are concentrated in the central and southern parts of India, mainly in the states of Madhya Pradesh, Gujarat, Rajasthan, Andhra Pradesh and Karnataka. Among the different oilseeds produced, groundnut, rapeseed - mustard and soybean together combines for about 80% of oilseeds area and 87% of oilseeds production in the country (2008-2009) meeting its domestic edible oil requirement. India's import bill for edible oils was more than Rs 26,485 crore during 2009-2010. **(Agricultural Statistics at a glance, 2010)**.

The rise in the amount of edible oil imported also shows an increasing trend. Import of edible oil increased at an alarming rate from 4.9 Mt in 2007-08 to 8.1 Mt during 2009-2010.Continous dependence on other countries for import of edible oil results in a significant drain of foreign exchanges and fund reserves of the country **(Mittal, S., 2018).** The lack of availability of edible oils in the country and increase of import duty had made it difficult for meeting the rise in demands and has to depend on foreign nations for the supply **(Dohlman *et al*., 2003).**The per capita consumption of edible oils is at 12.7 kg/ annum and it is well below the world average of 23.46 kg/annum. Imports of edible oil was being kept under lower restrictions and this led to liberalization of edible oil during 1996-97. The policy of liberalization stemmed as much from the lack of self-sufficiency in domestic edible oil production under the commitments as it has been established under the new multilateral trade regime under **WTO (World Trade Organisation).** India is the storehouse for oilseeds as no other country in the world has shown the cultivation of the range of annual and perennial oilseeds that grows in different agro climatic zones **(Malaviya, R. and Yadav, N., 2020).** Besides the rapeseed and mustard seeds oils have also been extracted by technological process from Rice Stem Bran, Cotton seed, maize germ etc. In addition to these, seeds of some forest trees and tree species are also being

exploited for vegetable oils. Apart from being the essential part of human diet these oils has served as an important raw material for the manufacturer of soaps, paints, varnishes, hair oil, lubricants and textile auxiliaries **(Shahidi, F. ed., 2005).** Although India has 20.8 percent of the world's area under oilseeds crops,it accounts for less than 10% global production **(Mitchell, D., 2008)**.This is because of the flow productivity of oilseed crops and year to year fluctuations in production which could be attributed to the following limitations and the bottle neck **(Sharma *et al*.,2010)**. Having limited scope of bringing additional area under oilseeds, increase in the production of oilseeds has primarily come from land saving and also establishing technologies highlighting a combination of high yield plant type, standard crop management practices and balanced crop nutrition. (**Antle, J.M., 1999).**

India is considered as a utopia for oilseeds as no other country has the range for the annual and perennial oilseeds grown in different agro climatic zones (**Singh *et al*., 2013**). The edible oils can also be extracted by using technological process from rice bran, cotton seed, maize germ etc. Inspite of this seeds of some forest and tree species are also being used for extraction of vegetable oils. These oils obtained also form an essential part of human diet as it also serves as an important raw material for the manufacture of soaps, paints and varnishes, hair oils, lubricants, textile industry pharmaceuticals etc. The low productivity of oilseed crops and year to year fluctuations of production in India that generally leads could be attributed to the following constraints and bottlenecks**. (SHODHGANGA**).Oilseeds apart from being sources of oil has a lot of importance as nutritious food items. Oilseeds like soybean, groundnut and sesame are used as food and in the manufacture of various value added and nutritious food items (**Nevara *et al.*, 2022**).The components of the oilseeds like oil, protein, carbohydrate, minerals, vitamins and trace constituents have huge and diverse uses. As studied animal products have a negative impact on human health as it increases the blood cholesterol levels

and causes obesity. Hence their usage is under review.Ethical considerations and cruelty against animals also go against the use of animal products **(Nagaraj, G., 2009).**

The cultivation of oilseeds in India is a major concern because they provide uncertain returns on investments and is a major problem to the country. They are mostly grown in those areas having scanty or no rainfall along with poor soil health .This has led to the scarcity of domestic oilseeds **(Singh, R.B., 2000)**. The previously evolved varieties have not led any results, and thus the previously led forms are just out of any use. There is a lack of supply of quality seeds due to constraints in their large scale production .Farmers are also hesitant to allow improved varieties of seeds as it requires high doses of fertilizers and pesticides ,which rather increases the cost input (**Chauhan *et al*., 2012)**. Thus there is a kind of virtual inactivity in the yield levels of most oilseed crops.

At present in the country the scenario is different, as it leads to take urgent measures to ramp up the oilseeds production on a sustainable basis since the growth in oilseeds production has not been able to keep pace with their increasing domestic demand. Exploiting the emerging technology and intensifying the use of land seems to be the feasible options. Alongside the use of improved technologies and educating the farmers by demonstration through media or any other medium should be incorporated to get higher recovery of oils and higher recovery of oil through efficient processing methods **(Rao, C.H. and Gulati, A., 1994)**. Also oilseeds and their various products needs a profitable and improved domestic marketing **(Reddy, A.A. and Bantilan, M.C.S., 2012)**. To cultivate this crops and make it economically superior and cost-effective so that the mass population can reach out to get the benefits, superior technology is needed that could yield boosting technologies. As there is a high demand on agricultural land from various crops and enterprises, the production of oilseeds can be increased significantly to reach the demand of the population by providing the farmers with newer technologies and they itself get the remunerative and attractive prices, easy availability of market access for selling

the crops. Inspite of these farmers has to face various restrictions in oilseeds productions. Most of the oilseeds are grown under rainfed conditions and only 25 percent area under oilseeds are irrigated area **(Raju *et al*., 2010)**. There are several factors that come into play that inhibit the exploitation of the yield potential of crops and those factors need to be solved which are likely such as biotic, abiotic, technological, institutional and socio-economic limitations. There is a large number of high yielding varieties or hybrids and production technologies that have been developed by ICAR, Agricultural Universities and a large number of public and private institutions all over India but still there is a deficiency of large range of high yielding varieties/ hybrids and production technologies that could give a huge amount of yields under rainfed conditions and these crops can also resist to insects, pests and diseases **(Pooniya *et al*., 2015).** Still there is a lack of improved farm implements low cost technology for control of insects, pests and diseases, appropriate post-harvest technology to prevent from post-harvest losses and decline in quality. **(Sharma, V.P., 2014).**

India is conferred with a number of oil yielding species of crops that includes those growing annually or perennially**,** and these crops are minor oil bearing species of forest and tree origin (**Murphy, D.J., 2014**). They are actually components of some non-traditional sources of edible oil such as rice bran, cotton seed and maize. India has a diverse agro-ecological areas for the growth of these oilseed crops and cultivation of these crops do not require huge labour source **(Jat *et al*., 2019)**.Scarcity or shortage of labour condition doesnot provide any decrease in production as these crops can be handled in such scenarios. The cultivation is very economical and remunerative and thus helps in improving the socio-economic status of the farmers. A related but diversely different restriction associated with oilseed farming is that some of the best available varieties do not find approvals at the state level. The cause of the problem lies generally in the existing weak research-extension linkages for the oilseed crops at the national and state level. The alleviation of this particular restriction should be given utmost importance

in any programme that aims to increase the production and productivity of oilseeds crops in the country.

According to the recent statistical data rate the country has about 2,50,000 ghanies;15,000 power mills;50,000 expellers and about 400 solvent extraction plants for processing the oilseeds **(Mathew, E.T., 1960).**The scientists have identified non-conventional areas and seasons for upholding oilseed cultivation with the availability of newer technologies and it is expected that in the near future there would be diversions of area from some of the traditional crops for the cultivation of oilseeds. The experimental evidence available from the research in crop and cropping system in recent years suggest that through introduction of restructured varieties of annual oilseed crops in the place of the traditional cropping systems, a wide range of niches and restructured cropping patterns not available so far could be set up.

As the standard of living is becoming modern along with flourishing economy due to the industrial growth and newer technology in agriculture and food development industries the demand for the vegetable oils is also rising at a rapid pace. The global market for vegetable oils is projected to exceed 275 million metric tonnes by the year 2024 (**Bose *et al*.2021**).This demand is appearing based on the growing consumer interest in healthy, organic and unprocessed/ unrefined vegetable oils. The global vegetable oil market is dominated majorly by palm oil that contributes for more than one-third of the total vegetable oil consumption **(Mielke,T., 2018).** Palm oil is followed by soybean oil, canola oil and sunflower seed oil and it has been produced at a huge amount to meet the demand. But according to the World Health Organisation (WHO) the use of palm oil causes serious health effects such as cardiovascular disease and many more **(WHO 2003)** . At present, there is a great demand of vegetable oil internationally, but alternative sources are a few. Therefore, it is necessary to find alternative sources of vegetable oil from underutilized oilseeds resources to fulfill the global scarcity of vegetable oil applying non-agriculture land **(Shi *et al*., 2019).**

*Sterculia foetida* oil is one such type that can be considered as an alternative sources of edible oil which can help in fulfilling the demand of edible oil in India .*Sterculia foetida* was first described in 1753 by Carolus Linnaeus. It is a soft wooden tree that grows upto a height of about 115ft.The name Sterculia genus originates from Sterculius, the Roman God of fertilizer or manure, and belongs to the family Sterculioideae of the family Malvaceae. Previously it belong to the subfamily Sterculiacea comprising of approximately 200 species distributed in the major parts of tropical and subtropical regions(Phytochemistry, biological activities and economical uses of the genus *Sterculia* and the related genera**. (El Sherei *et al*., 2016).**

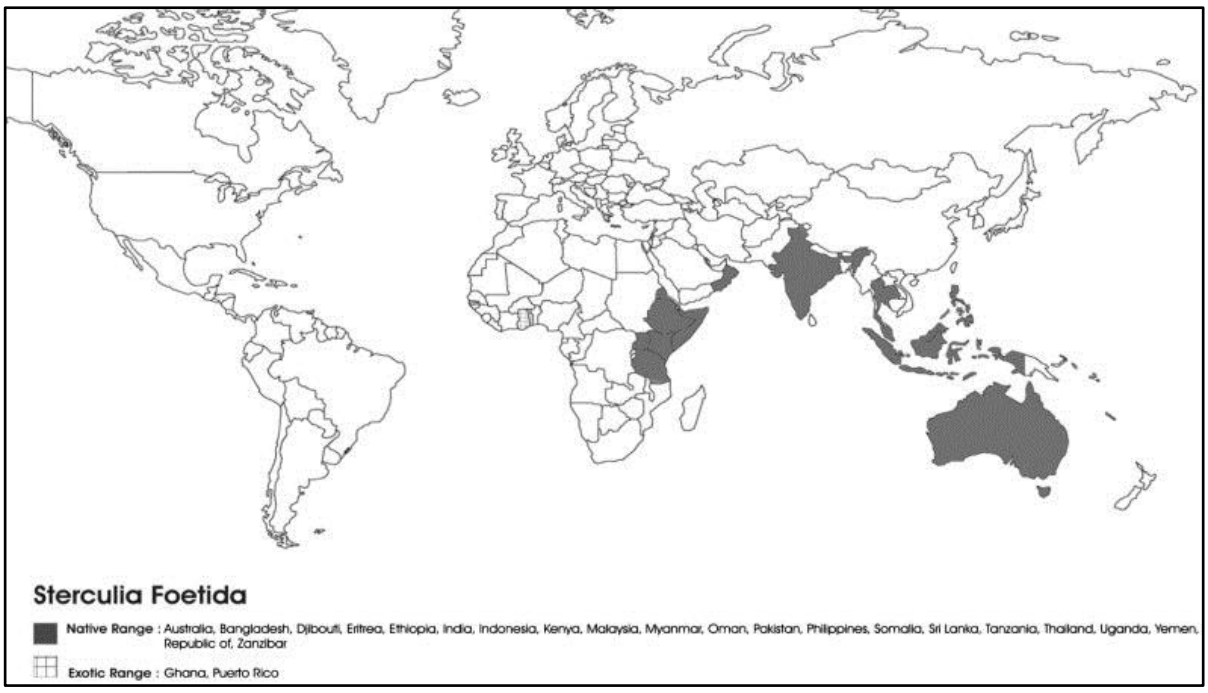



**Figure-1 - *Sterculia foetida* trees present in these countries**

Distribution map of Sterculia foetida, L.
(Source: www.worldagroforestry.org)

These plants are natural inhabitant from East Africa to North Australia and also grows freely in Myanmar and Srilanka etc. and to some extent in India. It is also unusually distributed in Ghana and Puerto Rico.

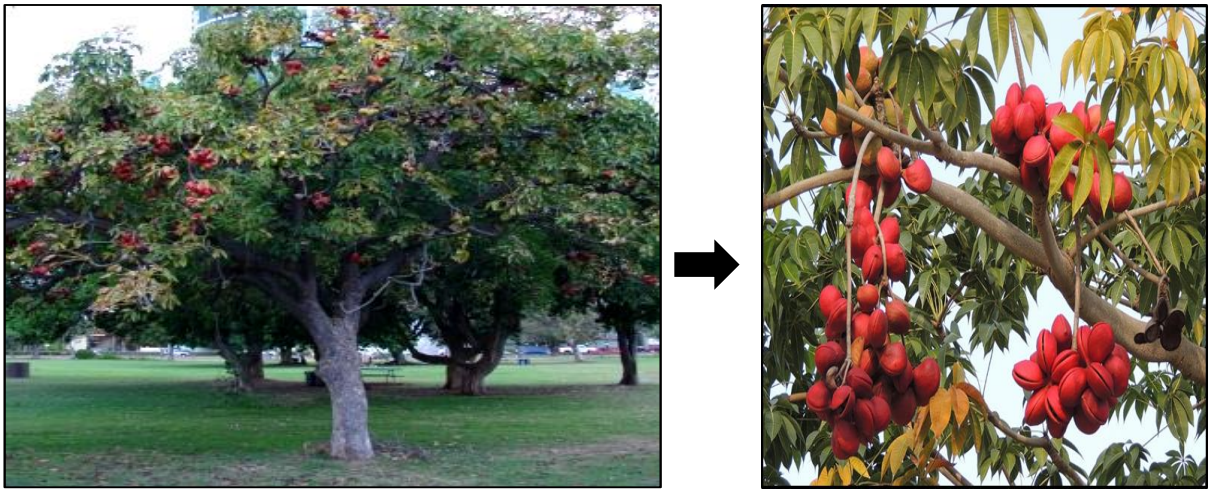


**Fig:2 *Sterculia foetida* tree, Family- *Malvaceae*, Subfamily- *Sterculiacea*, Genus- Sterculia,Species- *foetida* .**

*Sterculia foetida* commonly known as bastard poon, java olive , hazel sterculia ,wild almond is a large straight deciduous tree **(Raj *et al*., 2022).** This plant has a life span of more than 100 years. The plant grows rapidly and produces seeds within 2-3 years and the productivity is reported to approximately 2000-2500 kg of seeds from one tree per year. Socymbium R.Brome of the *Sterculia* species are classified under different genera based on distinct morphological features which are Pterygota Schott and Endl; Firmiana Marsili, Brachychiton Schott and Endl. Hildegardia Schott and Endl. Pterocymbium R.Br and Scaphium Schott and Endl ; Karaya or Indian gum which has been extracted from *Sterculia urens* Roxb (S.urens),used as a thickener and emulsifier in foods, as a laxative and as a denture adhesive **(Dhiman *et al.*,2019).** Some species from the genus *Sterculia* were used for the production of timber and also cultivated as ornamentals purpose. The genus *Sterculia* contains various classes of compounds including flavonoids and their derivatives, terpenoids mostly as triterpenoids, coumarins alkaloids and other classes such as phenolic acids, phenyl propanoids, fatty acids, sugars and some steroids **(Al Muqarrabun *et al*., 2015).** *Sterculia foetida* seeds can be eaten raw or roasted and doesnot possess any harm to humans and animals. A variety of active compunds such as quercetin, epigenin and scopotin have been isolated from *Sterculia foetida* leaves. Sterculinine-I,sterculinine-II and soya cerebroside-I were isolated from the *Sterculia lychnophora* seeds.

**Fig:3(a)**

**3(b)**

**3(c)**

**3(d)**

**Fig:3(a),(b),(c),(d)- Flowers of *Sterculia foetida*, leaves of Sterculia and seeds of sterculia.**

An unusual feature of the *Sterculia* seed is that the oil is present in the testa as well as the kernel upto an extent of 45-50%. *Sterculia foetida* oil contains cyclopropane fatty acids namely sterculic and malvalic acids around nearly (50-55%)**. (Bindhu *et al*., 2012).**

The importance of the oil as a value added product has not been explored much .Very few literatures has given the reports on the use of *Sterculia foetida* oil to develop a variety of products. Branched chain fatty acids from the oil were used to develop lubricant base stocks. The studies on the phospholipids of *Sterculia* oil shows the cyclopropene fatty acid chain that were subjected to oxidation to prepare intermediate. However there exists no report on the use of this oil as biodiesel and comparing its properties with other biodiesel .This tree has immense potential for many medicinal applications. The leaves of *Sterculia foetida* contain up to 2.66%

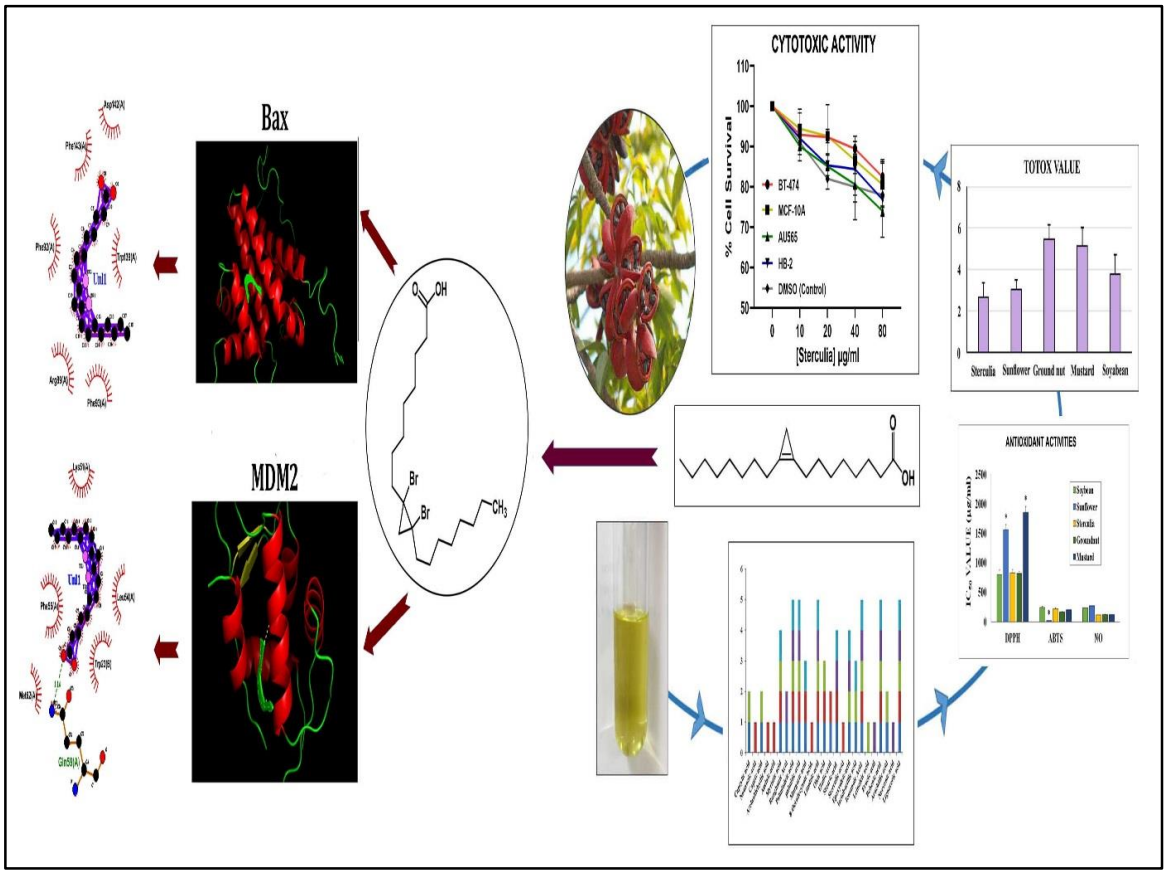
calcium and are also a good source of protein and phosphorus (**Prakash, Y.G. and Kaviarasan**, **L.G.V., 2012).** The leaves of this plant have been reported to have various pharmacological properties including laxative, carminative, anti-inflammatory, antioxidant, antimicrobial, cytotoxicity, anti-diabetic, anti-hyperlipidemic and insecticidal activities (**Naik *et al.*, 2004**; **Hussain *et al.*, 2014**; **Mujumdar *et al.*, 2000**; **Vital *et al.*, 2010**; **Rani & Rajasekharreddy 2010**; **Suganya *et al.*, 2017**). Methanol extract of *Sterculia foetida* seed possess antioxidant activity **( Galla, 2012).** The de-oiled seed cake was reported to be rich in protein (28-89%) that can be used as animal and fish food supplements **( Oliveira, *et al.*, 2000**; **Shamsundar & Paramjyothi, 2010)**. The oil constitutes of 7 fatty acids whose carbon chain length, degree and position of unsaturation were determined from the characteristic ionization and fragmentation of FAME resulting from GC-MS electron impact, chemical ionization modes. The fatty acid found in the oil were methyl esters of Tetradecanonic acid (Myristic acid) (1.65%), Hexadecanoic acid (Palmitic acid) (11.87%), 9-Octadecenoic acid (20.50%) [7], 10-Octadecadienoic acid (Linoleic acid) (20.50%), 8-(2-Octacyclopropane-1-yl) octanoic acid (Sterculic acid) (6.76%). It also contains Isocutellarin, Procyanidin-B-D-glucuronide, 6-O-B-D glucuronyl leteolin and cyanidine-3-O-glucoside isolated from the leaves. Leucoanthocyanidine-3-O-alpha-L-rhamnopyranoside and quercetin rhamnoside isolated from root. **( Bindhu *et al*., 2012).**

The first objective of the work to be done was to validate the capability of *Sterculia* seed oil as an alternative safe edible vegetable oil compared to other edible oils by determining oil and FAME content in the seeds of Sterculia foetida, L. along with commonly used edible vegetable oilseeds namely Sunflower, Groundnut, Mustard and Soybean. Next proximate composition analysis of the Sterculia seeds along with other edible oilseeds was performed by determining the mineral composition and analysis of dried seed flakes of Sterculia along with Sunflower, Groundnut, Mustard and Soybean seeds.Quality assessment with other edible vegetable oils

based on fatty acid composition, antioxidant activity and oxidative stability was also done. Totox value, Iodine value of the five seed oils of Sterculia, Sunflower, Groundnut, Soybean and Mustard was also performed. Toxicity assessment test was done by carrying out MTT assay.

And final objective of my study was to extract sterculic acid from the sterculia oil which is a cyclopropene fatty acid present in the seeds of *Sterculia foetida*. Previously there has been some reports that showed the halogenated compounds are potent anticancerous agents with target specific activity **(Lu et al., 2015; Kubanik et al., 2018; Trippier et al., 2020)**. Therefore, we are interested to synthesize a halogenated derivative of sterculic acid by attaching halogen atoms in the cyclopentene ring that would biologically more active than sterculia oil and also have good anticancerous activity.This sterculic acid was reacted by brominating to break the cyclopropene ring to synthesize a new bromo product of this acid by joining two bromine acid compounds in that ring which acts as an adjuvant. This adjuvant has been found to show more positive response in case of antimicrobial activity and anticancer activity. As it is target specific so it can be applied to the particular site of infection or cancer cells without even hampering the normal cells. This new natural synthetic compound can be used for targeted drug delivery for its site specific mode of action.Thus in this study, an in-depth characterization of fatty acid composition of Sterculia seed oil and four other commonly available vegetable oils (Groundnut oil, Soybean oil, Mustard oil and Sunflower oil) along with their various phenomenal characteristics that include their oxidative stability, radical scavenging activity, proximate and element content and cytotoxicity tests have been performed to validate its use and marketing it as safe edible oil.

# Fig:4 Graphical Abstract



# Chapter-2

# REVIEW OF LITERATURE

**Biofuel** : Fossil fuel has been the main energy source for humanity (coal, oil and natural gas) that has been developed from the result of decomposition of many living organisms for centuries. Though the usage of this energy has several disadvantages such as

- Conversion of fuel into electricity, combustion of fuel for engine work gives out a lot of $CO_2$ emission to the environment that results in climate change (**Voloshin RA *et al*. 2015; Allakhverdiev SI *et al.*2015**)

- Harmful compounds such as carcinogens and poisons are released to the atmosphere while using petroleum fuels.

- Finally fossil fuel is getting exhausted at a rapid pace because the rate at which it is formed is incomparable with the rate of its consumption. Almost 80% of the consumed energy all over the world comes from the fossil fuels. In the field of electrification, hydraulic power industry in (Canada, USA and China) and nuclear power stations in (USA, France, Japan) significantly compete with fossil fuels.But both the systems seems to be connected with considerable risks of ecological and humanitarian disasters.( **Voloshin RA *et al*.2016; Allakhverdiev SI *et al*.2009, 2010; Razzak SA *et al*. 2013)**.

Thus nowadays, attention has been focused to the alternative energy sources that are developing day by day. Biofuel production is a promising trend of alternative energy, exactly in transport sector. It is a substance with large value of heat of combustion obtained from biomass. Proteins, lipids and carbohydrates are the main components of dry biomass. Not all the biomass is used for fuel production.

Three main components of biomass are the precursors of biofuel **(Dragone G *et al.* 2010; McKendry P *et al.* 2002)**.

(a) Vegetable oils or animal fats.

(b) Starch and oligomeric sugars

(c) Lignocellulose.

For biodiesel synthesis fats are the most important component. Alcohols and biohydrogen are obtained from starch and sugars via fermentation process (**Alonso DM *et al.*2010**).Though from long times people have learned to use biomass to satisfy the energy needs. The usage of biofuel in engines has been considered since the concept of mechanical engineering. Biodiesel has been considered as the first fuel for diesel engines instead of petroleum and diesel. Biofuel production could not be competed with cheap fossil fuels as this was easy to extract. For this reason Petro chemistry have become the main criteria in fuel power industry. But at present as there is increase problems due to the use of fossil fuels and also fossil fuel is decreasing day by day, then this environmentally friendly biofuels should be a good substitute for fossil fuels as it would become more popular (**Nada EM *et al.* 2011**). Biofuels has three general advantages in comparison with fossil fuels:

- Biofuel can be a renewable resource.
- Fewer toxic compounds are released into the atmosphere during the combustion of biofuel.
- There has been less or no $CO_2$ emission observed into the atmosphere.

**Biofuel generations:** Biofuels are generally divided into primary and secondary ones **(A.Demirbas.2009)**.The primary biofuel is the biomass that is obtained from the wood, wood chips , animal fats, residues of forest and agricultural crops. These are mainly used for heating

cooking and agricultural needs specially in the Third World countries. The secondary biofuel is produced from biomass (primary biofuel) by the extraction of the most resource-intensive substances such as bio-hydrogen, bio-methanol, biodiesel. This substances can be used as a proper substitute to fossil fuel.

These secondary biofuel can be divided into three generations-

**First generation , Second generation and Third generation (DM Alonso *et al*., 2010**;Energy education, 2020**)** which is based on the general feed stock for fuel production, and the sequence by which it is present in the world energy market.

***First generation*** : First generation biofuels are those fuels made from food crops grown on arable land. The crops sugar, starch or oil content is converted into biodiesel or ethanol using trans- esterification .

***Second generation*** **:** Second generation biofuels are produced from lignocellulosic biomass. Lignocellulose is present in high amount in plant biomass, which is the main component of cell walls. Lignocellulose consists of three components : cellulose (40-50%), lignin (15-20%) and hemicellulose (25-35%). These components plays an important role for the mankind, but the process of extraction is rather very tough especially that of cellulose. Cellulose is a rather straight glucose polymer with certain rigidity that impedes its hydrolysis **(Ng *et al*., 2015).** The feedstock used to make the fuels either grow on arable land, but they are the by products of the main crop because they are grown on marginal land. These feedstocks include straw, bagasse, perennial grasses, jatropha, waste vegetable oils, municipal solid waste and so forth.

***Third generation*** :The third generation of biofuel is connected with algal biomass .The use of algal biomass for generation of fuel evolves a new direction of bioenergetics. Algal biomass accumulates considerably high amount of lipids in comparison with biomass of oil plants. This fact considers algae as a good source of biodiesel production **(Downie *et al., 2019;* Devarajan**

***et al.*, 2021)**. The main difference between algal bioenergetics and plant bioenergetics is in the technology of biomass up-building. Plant bio-energetics requires the usage of arable lands and provides relatively low yield in a ratio of the organic feedstock mass to the mass of biofuel synthesized. Microalgae can grow in most adverse conditions which are unsuitable for plant growth, saline soils and waste water. So the usage of microalgae is being considered as an important potential feedstock for biofuels production **(Mata *et al*., 2010)**.

## Biofuels and the environment :

**Carbon neutrality**-

A biofuel project is said to be carbon-neutral if the $CO_2$ absorbed by the crop recompensate for the green house gas (GHG) emissons related to the project.The most important of all the greenhouse is $CO_2$ and approximately 27% carbon in $CO_2$ (12/44) is present. This includes any emissions caused by direct or indirect land use change. Many first generation biofuel projects cannot be called carbon neutral after this definition.

It is the total amount of absorption and emissions that together determines if the GHG lifecycle cost of a biofuel project is positive, negative or neutral. This can be assumed by determining that the emissions during production, processing, transport and combustion can be higher than what is absorbed , both above and below ground during crop growth, the GHG lifecycle cost is positive. Likewise, if total absorption is higher than total emissions the lifecycle cost is negative.

The increasing demand of fuel and fuel products together with change in climatic conditions led to the rising concern for energy crisis and environmental problems. Now biodiesel is the most favourable alternative fuel available for diesel engine from non-edible feed stock (**A.Abbaszaadeh *et al*.2012; M.Canakci *et al.*2008**). Biodiesel is defined as the mono-alkyl esters with long-chain of fatty acids derived from vegetable oils, animal fats or waste cooking

oil. It can be rendered as eco-friendly, non-flammable and non-toxic in nature. It also offers some more advantages than the common conventional petroleum and diesel available in the market. The most common advantages are bio-degradability, higher flash point, improved cetane number and produces less exhaust emissions (**Fazal MA *et al.*,2013**).Besides biodiesel is also free from sulphur or aromatic compounds and reduces air pollution like carbon-monoxides, hydrocarbons and particulate matter (**M.Balat,2011**).Thus for this reasons popularity of biodiesel rising day by day and gaining worldwide attention. The first generation biodiesel production from non-edible sources has come into the limelight as the yield of biodiesel is very high and it can be easily processed. However, edible oil based biodiesel faced the problem of fuel versus food debate and these factors negatively affect on the production of biodiesel from edible oils. Thus these non-edible vegetable oils and the second generation of feedstocks have gain more importance for biodiesel production. For non-edible feed stock for biodiesel production there has been various approaches to study the feedstock of *Jatropha curcas L* (**M.Mofijur *et al.*2012**), *Sterculia foetida L*. (**PK.Devan *et al.*2009**); *Calophyllum inophyllum L.*, (**Ong HC *et al*.2011**); *Ceiba pentandra L*.( **P.Sivakumar *et al*.2013**); *Nicotiana tabacum L.* (**AEM Abdelaziz *et al.*2013**); *Pongamia pinnata* **(KV.Thiruvengadaravi *et al*. 2012**); *Hevea brasiliensis* **(M.Morshed *et al*.2011)**; *Putranjiva roxburghii*, **(SK.Haldar *et al*.2009 )**. It is believed that the oils obtained from these plants can be one of the solutions to meet the world energy demand and to reduce the dependency on the edible oil.

## Healthy Edible oil :

Coronary heart disease (CHD) is the leading cause of heart ailment related disease all over the world **(Ghafoorunissa, G., 1994).** Its incidence is rising rapidly especially in developing countries such as India. Generally the edible oil plays an important role in the causation, treatment, management and prevention of CHD **(Asgary *et al*., 2018)**. Compared to the western countries, choosing the right edible oil for cooking is extremely important. High amount of

clinical trials and observational metabolic studies among diverse populations establishes a consistent association between quality/ quantity of fat intake and the CHD risk **(Hu *et al*.1997).** Edible oils have several fatty acids that can be grouped into three classes- saturated fatty acids (SFA) **(Hooper *et al*., 2020 )** (which have 3 groups, short chain, medium chain and long chain SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) that is further subdivided into linolenic (LC or n6), alpha-linolenic (ALNA or n3) acid; and trans-fatty acids (TFA**),** which are produced by hydrogenation of vegetable oils or marine oils (**Bach, A. and Babayan, V.K., 1982).**

In addition, edible oils also consists of several antioxidants (like tocopherols, oryzanol, carotenes, tocotrienols etc.) and along with it micronutrients. Saturated fatty acids are considered harmful for human health ,as they increases total cholesterol (TC) and LDL cholesterol a risk factor for atherosclerosis **(Vijayakumar *et al*.2016; .De Souza *et al* 2015).** Reducing the saturated fatty acid (SFA) from about 17% to about 9% of energy (RR 0.83, 95% Cl and 0.72-0.98) reduces the risk of CHD, assumed from the studies of randomized trials by meta-analysis.

A recent systematic review also establishes that the saturated fatty acid may not be as harmful as earlier. PUFA and MUFA are the other types of fatty acids that lower LDLc and are cardio-protective **(Jakobsen *et al*., 2009; Ristic-Medic, D. and Vucic, V;2013.; Vani *et al*., 2002).** It has been reported that substitution of SFA with PUFA significantly reduce the risk of CHD in a pooled analysis of 11 cohort studies. N6 (linolenic acid) and N3 (alpha linolenic acid) are the essential fatty acids that are required for proper functioning of the body. Not only LD is lowered by N6 PUFA but it also decreases HDL. Whereas N3 PUFA also lowers triglycerides, blood pressure, inflammation improve vascular function and sudden death. Both the N6 and N3 PUFA should be present in adequate and balanced proportion in the body that helps to compete for the enzymes that convert them into more active compounds **(ICMR. Nutrient**

**Requirements and Recommended Dietary Allowances for Indians,2010; AO/WHO**, **2010).**

Evidence suggests that in humans when omega intake is very low, plant based omega-3 is generally converted to long chain n3 fatty acids as is found in fish oils (eicosapentaenoic acid) in limited amounts. On the other side hydrogenation of vegetable fat (Vanaspati ghee) due to undesirable effects on serum lipids **(Joint, F.A.O., 2010)**, they are associated with an elevated risk of CHD that can cause even worse damage than the saturated fats **(Booker *et al.* 2008**; **Mozaffarian *et al.*2009** ). In several reviews it has been claimed that high intake of transfatty acids (TFA) was associated with increased CHD events and mortality and also related to other chronic diseases like Alzheimer's diseases, cancer, diabetes, obesity, inflammation, depression etc.**(Cicero *et al.*,2016**; **Budin *et al.*2009**; **Demonty *et al*. 2009)**.

Studies have shown that the intake of olive oil can confer to various health benefits in addition to reduction of heart disease risk **(Stark *et al.*2002 )**. Covas **(Covas *et al.*,2006)** has has demonstrated in a randomized controlled intervention that apart from monounsaturated fatty acids, its polyphenolic compounds usher a beneficial effects on plasma-lipid concentrations and brings about linear reduction in oxidative stress markers. However there are certain limitations to olive oil, of which the main limitation is that olive oil does not have ideal N6:N3 ratio and it may not be suitable for Indian cooking. Rather mustard oil is considered healthy edible oil because it is low in SFA, high in MUFA and PUFA, specially alpha-linolenic acid and a good N6: N3 ratio (6:5). It is also available in non-refined (cold-compressed) form that is relatively stable during cooking at high temperature. Mustard oil also associated with lower CHD risk as compared to other oils **(Rastogi *et al.*,2004)**. Another double-blind RCT has shown that in acute MI patients using mustard oil, there has been reduction in common symptoms of arrhythmias, heart failure and angina **(Singh *et al.*, 1997)**. Based on studies in rats, there was concern regarding high erucic acid content of mustard oil **(Charlton *et al.*,**

**1975)**, but later studies have reported that there is an inefficient activation of erucic acid to erucyl-CoA coupled with lowered activity of triglyceride lipase and enzymes associated with ß-oxidation of erucic acid that possibly contribute to the accumulation and retention of cardiac lipids. Low erucic acid rapeseed oil (canola) by virtue of its ideal LA/ALNA ratio , that has also been found to exert cardio-protective effects **(Lin *et al.* 2013).**

## Present Status of Oilseed crops and vegetable oils in India :

Oilseed crops are the second most important crop for the agricultural economy, which is next only to cereals within segment of other field crops. Self-sufficiency in oilseeds was attained through " Yellow Revolution" during the early 1990's but that was only for a short period of time. Being the fifth largest oilseed crop production hub in the world **(Karmakar *et al.*,2012)**. India is also one of the largest vegetable oil importers at present as there is a slight increase of oil consumption in respect of both edible as well as industry uses.

The demand-supply gap in the edible oils department has resulted in huge imports of oil that accounts for about 60 percent of the countries requirement .Inspite of huge growth of edible oilseeds in the country, there is still a high demand in the market (2016-17, import 14.01 million tonnes; cost Rs 73,048 crore) due to high galloping rate of per capita demand **(Singh *et al.*, 2019)** (~6%) and increase of per capita consumption (18 kg oil/annum) that is driven by increase in population.

### i) Common sources of vegetable oils :

**Primary sources of vegetable oil-** Nine oilseeds are the primary source of vegetable oils in the country. They are largely grown under rainfed conditions. Of these soybean (34%), groundnut (27%), rapeseed and mustard (27%) contributes to more than 88% of total oilseeds production. More than 80% of vegetable oil resource comes from mustard (35%), soybean (23%) and groundnut (25%) respectively. From the primary sources i.e the states of Andhra

Pradesh, Gujarat, Haryana, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal contributes more than 95% of total oilseed production in the country**(Narayan, P., 2016**).

**Secondary sources of vegetable oil –** Apart from these nine oilseeds, 3 million tonnes of vegetable oil is being obtained from secondary sources like cottonseed, rice bran, coconut, Tree borne oilseeds and Oil palm **(Sarkar *et al.*,2021)**. Palm oil which is considered as secondary sources of oils should be incorporated as the primary source **(Beyer *et al*.,2020)**.This is generally because it gives the highest per ha oil yield (4-5 t/ha).

**ii) Area, Production and Yield of Oilseed crops in India :**

In India the cultivation of annual oilseeds are done over 26.67 million hectares of area that produces about 30.06 million tonnes of oil seed crops annually **(Rathour *et al*.,2021).** About 70% of the oilseeds are cultivated under rain fed ecosystem. Presently, there has been a decrease in the production and output of oilseeds primarily because of the decrease in area under oilseeds, which is probably due to relative lower profitability against other competing crops like maize, cotton, chickpea etc. **(Gulati *et al*., 1997).**

**iii) Growth rate of oilseed crops :**

The growth rates of all annual oilseed crops during the last decade (2001-02 to 2011-12) is poor (in case of area and production) especially for sunflower, safflower, linseed, niger and negative for area of groundnut **(Narayan, P., 2016)**. Soybean and Castor crops showed a positive and high growth rates and rapeseed-mustard also registered higher rate of production.**(Kolar *et al*., 2020).** The annual production of oilseeds crops increasing at a good rate in the country and showed a positive growth during the period 2001-2013 as compared to the decade 1990-2000. The major factor behind these is due to the increased production that has come from the increase in area and highest rate of increase for productivity was bought

about by the use of modern technology **(Boserup, E. and Chambers, R., 2014).** Increase of area by 1.8 times, production by 4.99 times and yield by 2.86 times over 1966-67.

iv) **Import and export of oilseeds and vegetable oil :**

**Import –** Due to increase in demand India is heavily dependent on imports to meet its edible oil demand and India being the largest importer of vegetable oils (15% share) followed by China and USA **(Thapa *et al*.,2019).** Among the imported edible oils, share of palm oil is about 60%, followed by soybean oil with a share of 25% and sunflower 12% **(Lam *et al*.,2009).** Growth of import of edible oils during the last decade has increased by about 174%.

**Export –** As per the foreign trade policy export of edible oils has been restricted over the years. Currently the following rules are being followed.

a) Edible oils permitted for export in bulk as well as consumer packs of upto 5kgs with MEP.

1. Groundnut oil, sesame oil, soybean oil, rice bran oil and coconut oil.
2. Minor forest product oils.

b) Edible oils allowed for export in only consumer packs up to 5kgs with MEP of UDS 900 per MT.

India had never been a big exporter of edible oils (Olive oil, Palm oil, sunflower oil, safflower oil ,cottonseed oil, linseed oil and mustard oil) as India's export basket comprised of premium oils with higher value realization (refined coconut, groundnut and sesame oils) **(Dhole, S.S 2014).** Recently DOC oilmeals castor oil, groundnut and sesame oil contributes to the largest share in Indian economy.

**Vision :** In respect to the growing domestic demand for edible oils, the lurching deficiency and the cost to the exchequer on account of imports. There has been a new action planned by the Government to achieve a production of 45.64 million tonnes from 9 oilseeds crops by 2022

**(Sinha *et al*., 2018).** In 2013, it has been estimated that an additional production of about 15.58 million tonnes over and above the 30.06 million tonnes production is expected.

**v) Anticipated area, production and yield :**

The total vegetable oil requirement in the country in 2022 has been estimated to be 33.20 million tonnes taking in part the per capita consumption of more than 22kg/ person per annum. The anticipated vegetable oil production from 45-64 million tonnes of oilseeds in the year 2022 is 13.69 million tonnes. The vegetable oil availability from secondary sources such as coconut, cottonseed, rice bran, solvent extracted oil (SEO) (**Srivastava *et al*., 2021**) and with this tree and forest origin has been estimated at 5.22 million tonnes by 2022.

**vi) Strategies :** The strategies for enhancing the productivity and profitability of oilseed based production system has been prepared for meeting the annual oilseeds and for oil palm in the country. The strategies/ steps that has been proposed in oilseeds are time tested formula with scale neutrality that can be grounded for enhancing the productivity of the oilseed based production system with necessary institutional support (**De Bhowmick *et al*.,2019**).

**Proposed strategies are-**

1. Increasing seed production and distribution of newly released varieties.
2. Use of low-cost technologies with high impact on productivity that will result in higher income.
3. Technologies with high impact that involve reasonable investment with high return on investment (ROI), that emphasizes on eco-friendliness, input use efficiency.
4. Main step that emphasis on quality improvement and value addition leveraging technologies with a bearing on the employment through skill development.

5. Major emphasis on the development of newly other types of edible oilseed crops that is similar in function and credibility to the major oilseed crops (Soybean, Sunflower, mustard, groundnut) and can be produced in large quantity to avail for the masses.

Study suggests that palm oil is produced in large quantities and is followed by soybean oil, canola oil and sunflower seed oil **(Fry, J. and Fitton, C., 2010)** that has been produced to meet the market demand. But according to the World Health Organisation (WHO), palm oil consumption causes serious health issues such as cardiovascular diseases and many more serious health issues **(WHO 2003).** At present there is a great demand of vegetable oil universally but options are few. Thus there is a need to find alternative sources of vegetable oil from under-utilized oilseeds resources to fulfill the global scarcity of vegetable oil. Sterculia foetida seed oil is one such type that can be considered as an alternative sources of edible oil that help in fulfilling the demand of edible oil in India.

## **Sterculia foetida** –

*Sterculia foetida* L. is a tall deciduous tree belonging to the family *Sterculiaceae*. It is commonly known as Java olive, Poon tree, Wild almond, Hazel Sterculia and Sterculia nut (**Kavitha *et al.,* 2015**). In India it is commonly known as Jangli badam in Hindi and Gorapu badam in Tamil. It was first described by Carolus Linnaeus in the year 1753. The name Sterculia originated from the word *Sterquilinus,* Roman God who is also known as the God of fertilizer or manure (*Sterculia foetida* Linn.). It produces more or less whorled horizontal branches and the follicles are generally woody, boat shaped and bright red when they are ripe. The seeds are not winged but are black, ellipsoid, 2cm long, 10-15 seeds are present in each follicle **(Shamsundar *et al*.,2010)**. *Sterculia foetida* has enormous potential for its medicinal and economical importance. The gum can be used as controlled release matrix polymers **(Chivate *et al*.,2008)**. The seed crude extract of the plant can be used as an insecticide to Asian

army worm and also as anti-feedant to the semi-polar, *Achaea janata* (**P.Usharani and P.Rajasekharreddy, 2009)**. The oil of sterculia (3%) obtained from the seeds when applied in the diet has been found to delay the sexual maturity of the female rat as has been determined by the criteria of age at the time of vagina opened and the regularity and length of consecutive estrous cycle. Oil has also been found to treat nausea and common skin diseases **(Maurya, R. and Dongarwar,N., 2012).** The flavonoid content of the plant shows a good anti-oxidant activity **(Manivannan *et al*.2011)**.The seed extract of this plant can be used as a good insect repellant **(Chopra *et al*.1956).**This plant shows antifungal **(Schmid MK and Patterson GW,1988)** laxative, astringent, carminative, anti-inflammatory and central nervous (CNS) depressant activity **(Naik *et al*.2004)**. The locals in the villages often roast the seeds of this plant and is eaten as chestnuts. The seeds also has the potential for treating diseases like itch, skin disorder (**Policepatel, S.S. and Manikrao, V.G., 2013)** and rheumatism **(Shamsundar**, **S.G. and Paramjyothi, S., 2010)**. The phytoconstituents of the seeds constitutes of sterculic acid triglycerides (**Guerere *et al*.1985**), cyclopropenoid fatty acids that contains antifungal compounds, insecticidal, anti-viral, hormonal, antibiotic, carcinogenic or antitumour activities **(J.Salaun.2000).** After extraction from *Sterculia foetida* seeds the fractions and pure compounds obtained have good insecticidal activities against *Sitophilus oryzae* L., *Callosobruchus chinensis* L. and *Tribolium castaneum* **(Pathipati UR and Pala R, Journal of Pest Science,2010).**

## Taxonomic classification

**Kingdom** : Plantae

**Sub-Kingdom** : Tracheophyta

**Division** : Mangoliophyta

**Class** : Magnoliopsida

**Sub class** : Dilleniidae

**Order** : Malvales

**Family** : *Sterculiaceae*

**Genus** : *Sterculia*

**Species** : *foetida*

## Habit and Habitat :

This is a large, straight and deciduous tree that grows to a height of 40m from the soil surface. It is found mostly in lowlands, dry wood lands and throughout East Asia-India, Srilanka, Myanmar and Thailand. It is found at elevations of up to 1500m.

## Morphology :

**i. Leaves-** Leaves are crowded at the end of branchlets. They appear as digitate with 7-9 leaflets. These leaflets are elliptical lanceolate, acuminate and are 10-17 cm in length. Petioles are generally 12.5-23 cm long and they produce an unpleasant smell. Leaves generally consists of glucuronyl derivatives of procyanidin, scutellarein and luteolin and also consists of taraxerol, n-otacosanol and beta-sitosterol **(Orwa C, 2009).**

**ii. Flowers-** Flowers are present in enormous amount. They have numerous pannicles and are subterminal. These flowers are unisexual and 10-15 cm long with male and female flowers on separate trees. The calyx are generally dull, orange coloured and 1-1.3 cm long.

**iii. Seeds -** Seeds are generally present in huge amount.Their shape is ovoid-oblong, dark brown or black in color with a small yellow rudimentary at the base. It consists of cyclopropane fatty acids such as Sterculic aid and Malvalic acids that has various medicinal properties.

**iv. Bark –** The young bark is generally smooth and grey in colour and the older bark is dark brown and rough. Bark has a brown spot and faintly ridged. Thickness of the bark is about 25-30 mm (2.5-3 cm). It is more fibrous in nature **(Kavitha, M., 2016).**

**v. Root –** The roots are mostly thick and strong in nature and appears to be brown in colour. The roots are found to consist of leucoanthocyanidin-3- alpha-L-rhamnopyranoside, quercetin and rhamnoside.

## Traditional uses :

*Sterculia foetida* Linn has enormous important medicinal value and mostly all parts of the plant has its use for treating various diseases. Leaves-Decoction of the leaves has its use during difficult labor, as an aperient, diuretic, abortifacient and used to treat suppuratives cutaneous eruptions.It has been found that fresh leaf juice can be used as a good insect repellent. Fruit-Decoction of fruit is mucilaginous and used as astringent and in the treatment of gonorrhea and diarrhea. Bark –Decoction of bark is used in the treatment of dropsy and rheumatism. Also as aperient, diaphoretic and diuretic. Seed and seed-oil Kernels (seed) are edible when it is roasted and if eaten raw it shows it shows a good laxative effect. Seed oil that can be used internally and externally for the skin disease. The wood is boiled with seed oil and used externally in rheumatism **(Kavitha *et al*.2015; CP Khare,2008).**

## PHARMACOGNOSTICAL REVIEW:

**Taxonomic Significance of Petiole Anatomy of Sterculiaceous Species-**

Transverse section of petioles of *Sterculia foetida* showed collateral and open vascular bundle. The shape of vascular bundles are circular. It consists of 10-11 vascular bundles and are arranged in a circular fashion. The two accessory are present at the proximal part, 2-3 accessory vascular bundles are present towards periphery of pith or at the centre. Sclerenchymatous

patches of bundle sheath are present that consists of mucilage cavities present in few, random, within pith and cortex. Xylem and phloem are present in a uniform manner (**Mitra, S. and Maity, D., 2014**).

**Shoot Regeneration from Shoot tip Explants of *Sterculia foetida* Linn-**

Shoot tip rejuvenation were derived from the seedlings of *Sterculia foetida* using MS medium that is supplemented with various growth regulators and its combinations. Some of the growth regulators are cytokinins like N6-benzylaminopurine (BAP), Kinetin (KN), thidiazuron (TDZ) and auxins like indole acetic acid (IAA), naphthalene acetic acid (NAA) and indole butyric acid (IBA). Seedlings were raised like shoot tip, epicotyl and hypocotyl. For shoot elongation, regenerated shoots were placed on another MS medium containing IAA or IBA or NAA. The regenerated shoots were isolated and placed on root inducing medium for the formations of roots from the basal end of the micro shoots. Then the plants are acclimatized and planted in the fields **(Anitha S and Pullaiah T,2002).**

**PHYTOCHEMICAL REVIEW:**

The different active phytochemical constituents have been found to possess a wide range of activities, which may help in the protection against incurable diseases. The recent research on the leaves reported the presence of flavonoids, fatty acids, phenols and triterpenoids.

**PHARMACOLOGICAL STUDIES:**

Traditionally, *Sterculia foetida* is used in the treatment of various disorders by the traditional healers it is also used as aperient, diuretic, abortifacient, skin disease, anti-diabetic activity, anti-convulsant, antioxidant, CNS depressant and anti-inflammatory activity. The bark has been claimed to be used to treat arthritis which is not proven scientifically as yet. But it has many pharmacological activities of which some are as follows-

**1. Antidiabetic activity-**

The antidiabetic activity and anti-hyperlipidemic activity have been estimated in the extract of the leaves obtained by using methanol fraction of *Sterculia foetida*. Wistar albino rats. Glibenclamide used as standard. The extract was found to reduce the blood glucose level, cholesterol and triglycerides levels. Hence it is reported that leaf extract of *Sterculia foetida* showed significant antidiabetic activity and anti-hyperlipidemic activity which was comparable with that of the standard **(Kowallick *et al.*2014)**.

**2. Anticonvulsant effect-**

The ethanolic extract of *Sterculia foetida* leaves was testified to possess the anticonvulsant activity in pentylenetetrazole .The maximum electric shock induced convulsions were tested in albino rats. It exhibited significant anti-convulsant effect with high dose of 500mg/kg, decreases the duration of tonic clonic seizures, recovery time (131.2-5.02) and increases the percentage of inhibition of convulsions (68.09%) **(Raja *et al*.,2014).**

**3. Antidermatophytic Activity-**

The in-vitro antidermatophytic activity of petroleum ether and methanolic extract of seeds of *Sterculia foetida* were evaluated by Agar well diffusion method. The maximum anti-dermatophytic activity was found in methanolic extract when compared with petroleum ether extract against *C. albicans, T. rubrum, M. gypseum,* and *T. tonsurans*. It exhibited maximum antibacterial activity against *E. coli* and *B. subtilis* with streptomycin as standard. Hence, *Sterculia foetida* Linn., seed extract was found to possess antidermatophytic and antibacterial activity **(Bhusan *et al.*,2014 ).**

**4. Antioxidant Activity-**

The antioxidant activity of solvent extract of leaves of *Sterculia foetida* was evaluated by free radical scavenging, 1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide radical inhibition, the

inhibition of oxidation of beta-carotene, superoxide anion radical scavenging inhibition of xanthine oxidase activity methods. *Sterculia foetida* was found to act as a primary and secondary antioxidants. It scavenges free radicals and inhibit lipid peroxidation and have the beneficial effect on prevention of disease. The solvent extract showed significant antioxidant activity when compared to the standard drug **(Narsing Rao Galla,2012).**

**5. Antimicrobial Activity -**

The fruit extract of *Sterculia foetida* Linn. when combined with silver nanoparticles as silver nanoparticles have shown good antimicrobial activity and thus it is evaluated for their antimicrobial activity. Silver nanoparticles showed antibacterial activity against human pathogenic bacteria such as *Escherichia coli*, *Pseudomonas putida* and *Klebsiella pneumonia* **(Vital *et al.*,2010).**

**6. CNS Depressant /Anti-Inflammatory Activity-**

Alcoholic extract of leaves of *Sterculia foetida* have been tested on various animal models that shows significant anti-inflammatory activity. The extract exhibit significant activity in the acute carrageenan induced rat paw edema and the chronic granuloma pouch models. It's potentiality to be used as the barbital sodium and pentobarbitone that has the tendency to induce the sleeping time. Hence, the extract was found to possess CNS depressant and anti-inflammatory activity (. **AM.Majumdar, 2000** ).

**7. Anti-Obesity Activity-**

It has been reported that the oil extracted from the seeds of *Sterculia foetida* helps in reduction of belly fat and protection against obesity-related problems. The oil contains fatty acids which inhibit the action of an enzyme associated with insulin resistance, which indirectly reduce fats in the belly. It has the potential to be recognized as a natural herb for developing a good nutritional supplement **(James Perfield,2006).**

**8. Toxic and Anti-feedant activity-**

Acetone extract of seeds of *Sterculia foetida* Linn. has been studied for anti-feedant activity. Evaluation of anti-feedant and toxicity activity of crude extract of *Sterculia foetida* Linn. against the *Achaea janata* L., *Spathodea litura* F., and semi looper was carried out that showed its use as a good potential insecticide . The results were reported that the seed extract of *Sterculia foetida* Linn. can be used as a capable insecticide to the *S. litura* F. and *A. janata* L. respectively **(Usharani P and Rajasekharreddy P,2009).**

**9. Anti-fertility activity-**

Delay of sexual maturity of the female rat is treated by *Sterculia foetida* oil. The oil contains sterculic acid which improves the delayed opening of vagina and regularize the estrous cycle by degeneration of the membrane covering the vagina and by lengthening of estrous cycle according to the vaginal smear. *Sterculia foetida* oil was reported to be used as a good anti-fertility activity **(Sheehan *et al*., 1965)**.

**10. Bronchodilator activity-**

From the aqueous extract of stem bark of *Sterculia foetida* Linn., there is present this bronchial smooth muscles relaxant property. Preliminary studies of stem bark extract of *Sterculia foetida* reported that it stimulates the bronchial smooth muscles beta adrenoceptor, with an inhibitory effect on bronchoconstrictor like histamine, etc. Moreover it has been reported that stem bark extract has a type of antiasthmatic effect **(BK.Noamesi, 1986).**

**11. Mitogenic activity:**

Sterculic acid was isolated from *Sterculia foetida* oil, it was found to have mitogenic effect on the pancreas of male Sprauge Dawely rats. This mitogenic effect was due to the presence of cyclopropenoid fatty acids of *Sterculia foetida* Linn **(Scarpelli DG,1974).**

**PHARMACEUTICAL REVIEW:**

**1. Bio-diesel-**

From *Sterculia foetida* oil, biodiesel was prepared by using sodium hydroxide as catalyst and it has been evaluated for physico-chemical properties. The properties were confirmed with other oils present in the market used for biofuel and reported to *Sterculia foetida* Linn., as one of the non-edible feedstock for biodiesel production **(Bindhu *et al.* 2012).**

**2. *Sterculia foetida* Linn. Gum as Natural Mucoadhesive Polymer-**

A new in-situ muco adhesive nasal gel formulation had developed using a natural muco-adhesive polymer obtained from bark of *Sterculia foetida* Linn. The reports justify that barks of sterculia has been successfully used as a muco-adhesive natural polymer that was developed from *Sterculia foetida* gum. It is also used for the treatment or management of migraine for long period of time by sustaining the drug release **(Mahakalkar *et al.* 2013**).

**3. Evaluation of Gum as Controlled Release Excipient-**

*Sterculia foetida* gum (SFG) is obtained from the gummy exudates of stem bark of *Sterculia foetida* Linn. Various experimental parameters were evaluated such as characterization of SFG, compression of tablets, swelling studies, effect of SFG particle size, effect of SFG concentrations, effect of fillers, *in-vitro* release testing, effect of pH of dissolution media, effect of rotational speed, differential scabbing calorimetry (DSC), kinetic treatment, comparison of SFG with HPMC K15M. Reported to has greater influence on release rate of drug from SFG matrix due to higher concentrations of gum and rotational speed. It also proved to be better than HPMC K15 polymer in controlling the drug release **(Chivate *et al.*, 2014).**

# Chapter-3

# Materials and Methods

**A) Sample collection**- *Sterculia foetida* seed samples were collected from the campus of Indian Statistical Institute (ISI) ,Kolkata, India and also from the nearby areas of Kolkata in the month of December to January because during this time maximum number of seeds are obtained. Seeds have also been collected from the same *Sterculia foetida* plant present in other areas of Kolkata. Being a metropolitan city present in the eastern part of India and capital of West Bengal (22° 33' N , 88°20' E) in the Ganges Delta. The climate of this region is generally tropical wet and dry with scorch summer and wet monsoons.**(**"**Hazard profiles of Indian districts" National Capacity Building Project in Disaster Management . UNDP on 19 May 2006)** . The annual average temperature generally hovers around in the area of 26-30°C and the annual rainfall is around 1850 mm (73 inch). (**Weather base entry for Kolkata, Canty and Associates ,Archived from the original on 7th September, 2011**)**.** It lashes around Kolkata and southern parts between June to September. The city receives 2,107 hrs of sunshine on average per year with maximum exposure in April and May**.** (**Niwas *et al*., 2006, Textbook on Agricultural Meteorology)** The soil and water of Kolkata are predominantly alluvial in origin and it is based on the Indo- Gangetic Plain. It is situated on the lower Ganges Delta of Eastern India, the city's elevation is 1.5-9 m (5-30) ft . ( **Space radar image of Calcutta, West Bengal, India. NASA 15th April , 1999. Archived from the original on 14th Jan, 2012**.)

**B) Extraction of oil** : The seeds of Sterculia are dried by keeping at room temperature for days. From each tree nearly about 2000-2500 kg of seeds were collected. The seeds are cleaned to remove the dirt and the shell is removed manually (dehulled) and then grinned to powder, nearly finely powdered by using a Sample Miller Machine **(Cyclotec 1093,Sample**

**Mill,TECATOR)**.The ground fractions that passed a 500μm sieve (35 mesh) were used for extraction of oil (**Armah-Agyeman *et al*, 2016**).

100 gm of each grinded seed samples were soaked in 500 ml of hexane in 1000 ml capacity extraction flasks for nearly 24 to 48 hrs. After that the entire mixture was then vortexed at high speed to mix it properly and then mechanical stirrer **(Model No. - DC Stirrer NZ-1000s AC 220 V, EYELA )** was used for 2hrs at 3000 rpm for further agitation and mixing. After performing this the oil is extracted using a vacuum pump and filtered using a sintered disc funnel. The collected extract was concentrated in a rotary vacuum evaporator **(Rotavapor, R-3, BUCHI)** and the oil was recovered in the concentrating flask.

**C) Preparation of Sterculic acid** :

Sterculic acid a cyclopropenoid fatty acid found in the various plants that belongs to the genus *Sterculia*. It is the main component of the *Sterculia foetida* seed oil having a long chain ,monounsaturated fatty acid composed of 9-octadecenoic acid having a 9,10- cyclo propenyl group.It is derived from an octadec-9-enoic acid.

Preparation of sterculic acid is performed by undergoing saponification of the oil following the procedure of (**Salimon *et al*. 2012**). At first 50 gm of oil was taken and mixed in the reactor flask with 300ml. of saponifying solution comprising of ethanolic potassium hydroxide (KOH) concentrated and ethanol of about 300ml 90% concentration.The saponification was carried out in a 500ml round bottom flask with temperature controlled reactor at different temperatures of 50-70°C.This was continued for different times at a time of about 2.5-3 hrs till the saponification is complete. The change in colour confirms the saponification process. After saponification it is kept to cool down, then 200ml water was added and unsaponifiables were separated by extraction with hexane 100ml.The aqueous alcohol phase containing the soaps was acidified with HCL (6N) and the free fatty acids (FFAs) were recovered by extraction with hexane. The extracts were washed with distilled water to make it neutral (pH-7).Now the resulting lower layer formed was removed using a separate funnel and disposed away. The free

fatty acids present in the upper layer can be dried using anhydrous magnesium sulphate and solvent was evaporated in a vacuum rotary evaporator at 35°C.The free-fatty acid percentage and the fatty acid composition from the saponified oil sample was determined using Gas Chromatography fitted with Flame Ionization Detector **(GC-FID) (AOCS 1997).**

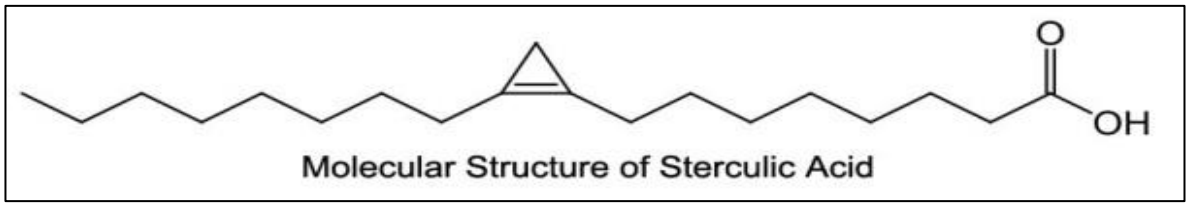



**Fig: 5. Molecular structure of Sterculic Acid**

**D) Preparation of Bromosterculic acid**

Bromination of sterculic acid is prepared which involves attaching the two bonds of bromine with the free end of the monounsaturated fatty acids composed of octanoic acid i.e 8-(1, 2-dibromo-2-octylcyclopropyl) octanoic acid.

At first 500µl of sterculic acid is mixed in 5ml of ethanol at room temperature.The samples are taken in a conical flask and is placed in a mechanical stirrer.Stirring is performed for 60-90 mins till the total acid gets dissolved in the solvent. After that bromine water is added dropwise in the stirring solution until a tint yellow colour develops. As the yellow colour is formed immediately adding of bromine is removed. Next the solution is poured on a petriplate and kept in open air to disperse the bromine vapour. It has to be kept in mind that bromine vapour is very obnoxious and poisonous to smell ,so it should be avoided in direct contact with the nose. After that the sample that is formed is applied on a Thin Layer Chromatography(TLC) plate and run on a solvent mixture of Ethylacetate:Ethanol (3:1) ratio and to compare its purity with another TLC plate where only sterculic acid was added and run simultaneously. Rf value showed clear difference between sterculic acid and newly synthesized bromo-compound. After this the samples have been sent to Central Instrumentation facilities of IIT-BOMBAY to

perform Liquid chromatography and Mass Spectra (LC-MS) to quantify and identify the new compound that has been formed by the reaction of the two chemicals.

**E) LC-MS analysis of newly synthesized bromo compound-**

For further confirmation, this newly synthesized bromo compound was subjected to LC-MS analysis (Agilent Technologies, MS Q-TOF, Model: G6550A; HiP Sampler, Model: G4226A; Binary Pump,Model: G4220B; Column Comp,Model: G1316C). The LC-MS analysis was done at the Sophisticated Analytical Instrument Facility, IIT Bombay, Powai, Mumbai-400076, India. Hypersil gold C18 column (100mm X 2.1mm, 3µm) was used and 5µl of sample (dissolved in hexane) was injected into column in the split mode for analysis at an injector temperature of 250°C. Solvent composition for Channel A is 100% water V.02 and 0.1% FA in water. For Channel B it is 100% Acetonitrile V.02 (90% ACN + 10% $H_20$ + 0.1% FA). Gradient run was performed for 30 mins. For first 1min gradient run for Channel A was 95% and for B it was 5%. Then for the $20^{th}$ min. it was 0.00% for A and 100% for B, for the next $25^{th}$ min it was again 0.00% for A and 100% for B. At the $26^{th}$ min.it was 95% for channel A and 5% for B. Finally at the $30^{th}$ min, it was again 95% for A and 5% for B.Pressure was 1200 bar and flow rate was 0.300 ml/min. The compound was identified by the mass spectra and molecular weight.

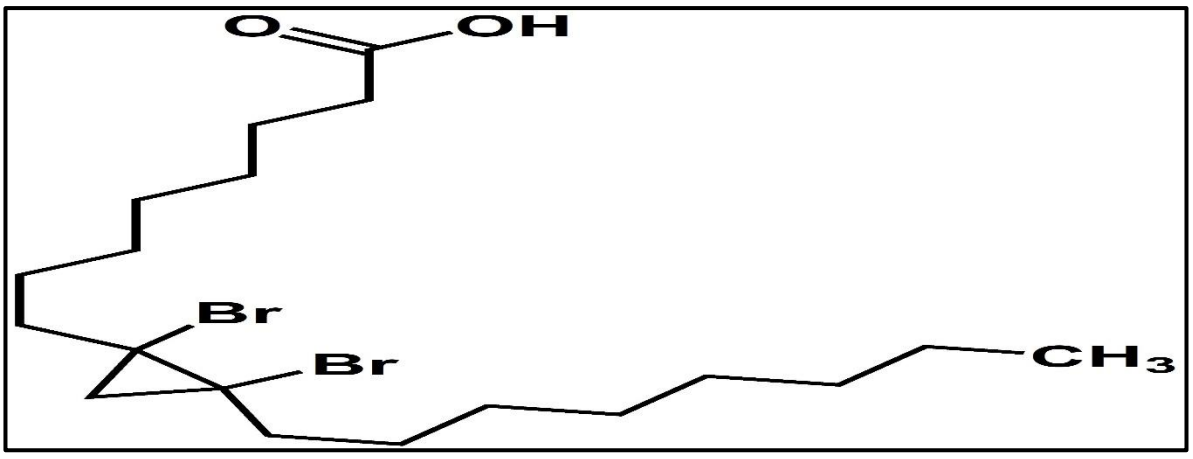



**Fig:6. Molecular structure of bromo-sterculic acid or 8-(1, 2-dibromo-2-octylcyclopropyl) octanoic acid**

**Procedure for the proximate analysis :**

**A. Methods to measure moisture content :**

To determine the moisture content of the oilseeds of Sterculia along with other oilseeds such as Mustard, Groundnut, Sunflower and Soybean, the seed flakes after extraction of oil using hexane or pentane or Petroleum Ether was dried in hot air oven at 70-80°C for about 7-8 days. During these days the weight of the seeds were measured every alternate day to find about how much the moisture has been removed and if there has been any leftover or if there is any change in weight of the seeds after those particular days. From the above method the percentage of moisture content can be obtained. [**AOAC 1980,Official methods of Analysis,(13$^{th}$ Edition) Association of Official Analytical Chemists,Washington DC)].** ( **Habib *et al.*, 2015)**.

**B. Methods to measure lipid content:**

Along with moisture the lipid content in seed flakes is also measured using the protocol "A Rapid method of total lipid extraction and purification" by following the **Canadian Journal of Biochemistry and Physiology,1959**.

Dilution with chloroform and water separates the homogenate into two layers, the chloroform layer containing all the lipids and the methanolic layer containing all the non-lipids. In this method the lipids of biological materials can be extracted and purified in a single operation. Mixture of chloroform and methanol had been used as a lipid extract and testing the mixture of the chloroform-methanol and water phase that led to the following method.This procedure can be applied directly to any 100 gm sample containing 80 gm water.It is important to keep the volume of chloroform, methanol and water before and after dilution in the proportions of 1:2:0.8 and 2:2:1.8 respectively.These ratios represent the total volumes present in the sample including the water present in the sample.Thus in the extraction of materials that has less than 80% water,either the size of samples can be adjusted so that they can consist of 80 gm of water

or 100 gm samples can be used and the volumes of chloroform and methanol are changed to give the correct proportions. Incase where the moisture content is much less than 80% it is necessary to add distilled water.

At first about 100 gm of sample is homogenized in a blender for 2mins and then the mixture of 100 ml chloroform and 200 ml ethanol is added and again 100 ml of chloroform is added to the mixture. The total mixture is blended for 30 secs. Into the mixture 100 ml of distilled water is added and the blending is performed for 30 secs. The homogenate is filtered through Whatman no.1 filter paper on a funnel with a slight suction. Filtration is normally quite rapid and when the residue becomes dry pressure is applied with bottom of the beaker to ensure maximum recovery of the solvent. Next the filtrate is transferred to 500 ml of graduated cylinder and allowed to separate for a few minutes for complete separation. The volume of the chloroform layer (atleast 150 ml) is obtained and the alcoholic layer is removed by rotary evaporator. The small volume of the chloroform layer is also removed to ensure complete removal of the top layer.The chloroform layer left behind contains the purified lipid.

**C. Methods to measure the protein content :**

It has been indicated in several papers that for the analysis of microgram quantities of proteins it is precarious to optimize Kjeldahl digestion to prevent loss of ammonia. The color changes are not so prominent for the Modified Kjeldahl Digestion method, but only a few micrograms of proteins were required to be digested. Here the fuming was clearly visible. Generally for about 10-15 mins digestion after the cessation of fumes is adequate to get optimum recoveries. Prolonging the digestion time generally results in solidification of the mixture and loss of ammonia. The total digestion times varies between 30 mins for pure proteins and 45 mins for proteins (**Wang *et al, 2016***).

Generally amount of protein content of organic matters can be determined by two ways-

i) directly by using certain specific chemical or physical properties unique to proteins.

ii) by indirectly determining their nitrogen content .

Nitrogen determination is the most commonly used procedure for a protein assay and it has been the primary method for various official and conventional methods currently in use for determination of total protein content of organic matters. The most commonly used nitrogen determination methods include various versions of **Kjeldahl (AOAC,1999a),Dumas (AOAC 1999 b) and combustion (AOAC,1999 c)** methods. Here Kjeldahl method has been most widely used for determination of nitrogen. The Kjeldahl method determines total nitrogen content and protein as the nitrogen content of the sample which is multiplied by a conversion factor . The sample is digested in sulphuric acid ($H_2SO_4$) using Copper sulphate (CuSO4)/ Titanium dioxide (TiO2) as catalysts , converting N to NH3 which is then distilled and titrated. This method is applicable to a wide range of organic matters including raw materials, ingredients and finished products from animals, cereals and oilseeds**.( Sáez-Plaza *et al.*, 2013).**

Nitrogen and Protein content of seed flakes can be obtained by three procedures-

Digestion > Distillation > Titration.

**Method** : About 200mg of dried powdered sample in 100 ml of micro-kjeldahl flask is taken .Then a pinch of catalyst powder ($K_2SO_4$ : $CuSO_4$ : $SeO_2$) in the ratio of 9:1:0.02 are added as a catalyst to speed up the reaction and make it more responsive. After that 4ml of conc. $H_2SO_4$ acid is added and the digestion of total mixture is performed until a light blue color develops. Then the reaction is stopped soon and allowed to cool and then transferred to a 50 ml volumetric flask. After cooling it down, the sample is then next transferred (about 5ml of the sample) in Markahms distillation apparatus and 5ml of 20% NaOH is added and distillation is performed. After the distillation ,the distillate is collected in 5ml of 20% boric

acid (Here it is to be noted that all the samples has been multiplied by 5 times to 25 ml,25 ml of NaOH, Boric acid has also been multiplied by 5 times to 25 ml ). Next the distillate is titrated against N/28 HCL acid, and by calculating 2ml of N/28 HCL acid is equivalent to 1mg $N_2$ which denotes that amount of nitrogen content present in 100mg of seeds. The amount of protein content can be assumed by multiplying the amount of $N_2$ with various conversion factors, such as for Sterculia it is 5.30, for sunflower also it is 5.30, groundnut it is 5.46 , mustard it is 5.30 and for soybean it is 5.71 respectively.

**D. Methods to measure the sugar content :**

Soluble sugar content is obtained by the official method for the estimation of carbohydrates using anthrone reagent. Carbohydrates takes part in an important role on the physiological activities of the plants and glucose and glycogen serves as an important source of energy for vital activities.

Carbohydrates are dehydrated with conc. H2SO4 to form “Furfural”, which condenses with anthrone to form a green color complex that can be measured by using colorimeter at 630 nm by using a red filter. Anthrone reacts with dextrins , monosaccharides, disaccharides, polysaccharides, starch ,gums and glycosides .

**Methods :** The sample is treated with 80% alcohol to remove sugars and then starch is extracted by reaction with perchloric acid. In hot acidic medium starch is hydrolysed to glucose and dehydrated to hydroxymethyl furfural. This compound forms a green coloured product with anthrone.

**Procedure :** At first about 100 mg of the sample is added into a boiling tube and is then hydrolysed by keeping it in a boiling water bath for 3 hrs with 5 ml of 2.5 N HCL. It is kept at room temperature to cool down. Now it is neutralised using solid sodium carbonate until the effervescence ceases. The volume of tube is made up to 100 ml and centrifugation is performed.

After centrifugation is performed the supernatant that is left behind is collected and from it 0.5 and 1ml aliquots are added for analysis. Now the standard is prepared by taking 0, 0.2,0.4,0.6,0.8 and 1ml of the working standard. Here '0' serves as the blank. All the tubes including the sample tubes are made up by adding the volume to 1ml with distilled water . Then 4ml of anthrone reagent is added . Now heating of the samples are performed for eight minutes in a boiling water bath. The temperature is cooled rapidly and the change of color from green to dark green is read in spectrophotometer at 630 nm. A standard graph is drawn by plotting concentration of the standard on the X axis versus absorbance on the Y axis. From the graph the amount of carbohydrate present in the sample tube is calculated. It should be noted that the contents of all the tubes should be cooled on ice before adding ice cold anthrone reagent.

**( Hedge *et al*., 1962).**

**Calculation** : The glucose content in the sample using the standard graph is obtained by multiplying the value by a factor 0.9 to arrive at the starch content.

**E. Mineral content of Seed flour :**

Any living organisms requires a continuous supply of huge amount of substance from food to reach their daily nutrient supply. This supply is called as nutrition. The mineral nutrition is an important asset and it plays an important role in human life for healthy growth. This nutrient present in the seeds is also an important source of nutrition for the cattles. It can also be used as fodder, along with several organic compounds as it has a rich medicinal value to cure diseases. The minerals may not play important roles in many activities in the body but these minerals are essential for human health such as ( P, K, Ca, Mg, Na, Cu, Mn, Zn, Pb) etc. These minerals are essential for both human health as well as for cattle fodder. Humans along with animals require a number of complex organic compounds as added caloric requirements to meet the need for daily physical activities for which mainly carbohydrates, fats and proteins

play an important role. While minerals and vitamins form a comparatively small part, they consist of the major portion of the diet as their nutritive value is important. (**Govindan *et a*l; 2015).**

**Sample preparation:** After extraction of oil from the seeds by using Soxhlet apparatus or by using mechanical stirrer by simple agitation, the seed flakes are obtained and they are then dried by placing it in an absorbent paperbag and kept in the hot air oven at a constant temperature of 85-90°C for 5-7 days ultimately to get the total moisture absorbed from it. After drying the seed flakes are taken in a mortar pastel and grinded to fine powder.

Here the presence of alkaline earth metals (Na,K,Ca,Mg) and heavy metals (Cu,Mn,Pb,Fe,Zn) in the seed flakes are obtained by using Atomic Emission Spectroscopy (AES) , **4200 MP-AES SYSTEMS manufactured by AGILENT TECHNOLOGIES**. Nearly 0.5 gm of the sample (seed flakes) dried in a hot air oven has been digested in the acid solution which combines of Nitric acid, perchloric acid and sulphuric acid in the ratio of 10:4:1 for about 6-8 hrs. This digestion procedure may vary from sample to sample depending on its composition and it seems to be complete when a pure clear liquid appears. The remaining volume is made up by adding distilled water to the volumetric flask and the following measurement are performed to find the amount of the macro and micro nutrients present in the seeds using the **Agilent 4200 MP-AES** .

All the five seed flakes of Sterculia, Groundnut, Mustard, Soybean and Sunflower are involved in this process and readings are taken in a triplicate manner to come to the ultimate conclusion.

**Mineral composition** –

Among the heavy metals, Sterculia seeds contained lowest amount of copper (16.67 mg/kg), lead (12.6 mg/kg), manganese (10.6 mg/kg), iron (938.67 mg/kg). Zinc (138.33 mg/kg) are the main metals in Sterculia as compared to other tested seeds. Highest amount of lead (48.67

mg/kg) and iron (146.33 mg/kg) were detected in mustard seeds whereas highest amount copper (52.67 mg/kg) and manganese (62.2 mg/kg) were recorded from groundnut seeds .

**F. Fatty Acid Profiling of Edible Oils :**

Fatty acids are the major component of lipids and the physical, chemical and physiological properties of a lipid class depending primarily on its fatty acid composition. The fatty acid composition is determined as the methyl esters of fatty acids by gas liquid chromatography (Gas Chromatography) is performed. Saponification followed by methylation is a classical method for preparation of fatty acids methyl esters (FAMEs) from glycerolipids and sterol esters (SEs). Base catalyzed methanolysis proceeds much more rapidly under mild temperature conditions than acid catalyzed reactions, and for this reason using KOH or NaOH catalyzed methanolysis completes within 2 min at room temperature for glycerolipids and within 1hr at 37°C for sterol esters.( **Ichihara, K.I. and Fukubayashi, Y., 2010**).

The analysis of fatty acids are very common in the edible oil industry and is usually performed by gas chromatography. Due to polar nature and the high boiling points of the fatty acids they generally shows poor peak shapes and bad reliability. A productive reliable data cannot be reproduced. To avoid these problem most method use derivatization reactions to convert fatty acids to fatty acid methyl esters (FAMEs), that are easier to separate and exhibit better peak shapes. The most common of the derivatization reactions are the base catalysed reaction which uses hexane and potassium hydroxide in methanol.

**Sample preparation-**

In a 5ml. screw top test tube approximately 0.1gm of the oil sample is weighed. Then 2ml of hexane solvent is added and shaked vigorously. After that 0.2ml of the methanolic KOH solution is added and the screw cap tube is fitted with a PTFE joint.The cap is tightened and shaken for another 30 secs. Then after shaking is performed the tube is left to stratify until the upper solution becomes clear.The upper layer is decanted containing the methyl esters . This

layer of the solution is ready for injection into the Gas- Chromatograph for analysis. Otherwise it can be stored in -20°C for future usage purposes (**Olive oil society**).

To compare the fatty acid profiling with respect to retention time of all the tested oils, fatty acid methyl ester (FAME) of edible oils were performed according to the AOAC official method 996.06 **(AOAC.2001)** with slight alterations described by Symoniuk .**(Symoniuk *et al.*, 2017).**

FAME samples of all the five edible oils were subjected to GC-MS analysis ( **Model No. Agilent Technologies, G-6860N Network GC System with 5973 inert Mass Selective Detector**) for determination of the fatty acids compositions. GC-MS analysis was performed at National Test House ,Salt Lake ,Sector V, Kolkata- 700091,India. Details of GC condition are: column type - HP-1MS (19091S-602); column (Length x I.D. x Film Thickness) - 25× 0.2 × 0.33; Injector type & temperature – SS, 280°C; Injection mode & volume – Split, 0.1µL, Carrier gas & flow – Helium, constant flow and 1ml/min. The oven temperature was programmed as follows: 50°C (1min hold), 50°C to 200°C at 7 °C/min, 200°C to 300°C at 6 °C/min, 200°C (2 min).The mass spectrometer was operated under the following conditions: ion source temperature, 280°C; ionization method, electrospray ionization (EI); ionization energy, 70eV; scan mode, full scan, scan range, 50-350 Da, acquisition rate – 0.2s. The total run time for GC/MS was about 30 min for each sample. The identification of FAME was done by comparing the mass spectra with the spectral data of the NBS75K library provided by Hewlett Packard with the GC/MS control and data processing software.

**G. Fatty Acid Composition of oils :**

For determining the fatty acid composition of all the tested oils, fatty acid methyl ester (FAME) of oils were prepared according to the AOCS Official Method 996.06 (**AOCS,2001**) with minor alterations described by Symoniuk (**Symoniuk *et al.*, 2017).**

FAME samples of all the five oils along with standard fatty acids were subjected to **MDLC analysis (Model No. WATERS Technologies, 2695, LC System with 2487 inert Mass Selective Detector**) for determination of the fatty acids compositions. MDLC analysis of the samples was done at Indian Institute of Chemical Biology, Jadavpur, Kolkata-700032, W.B. India. The gradient run was for 15 mins which was programmed as follows –First 0 mins for column A it was 100% and for B it was 0%, next 2 mins it was 100% for A and for B it was 0%, next 2.5 mins it was 80% and 20% respectively for column A and B. For next 4 mins,4.5 mins, 8 mins, 8.5 mins, 12 mins ,12.5 mins and 15 mins for column A and column B were 80% and 20%, 70 % and 30 % ,80% and 20% ,80% and 20%, 100% and 0% and 100% and 0% respectively. Total run was performed for 15 mins.

**H. Antioxidant activity :**

Antioxidant activity is defined as a limitation of the oxidation of proteins, lipids, DNA or other molecules that occurs by blocking the propagation stage in oxidative chain reactions and primary antioxidants directly scavenging free radicals , while secondary antioxidants indirectly prevent the formation of free radicles through Fenton's reaction. If a molecule in the process of charge loses an electron that it shouldn't then that molecule can become a free radical. It generally refers to unstable, electrically charged molecules that react and damage other molecules such as DNA.

Antioxidants react with these free radicals by taking the place of the missing electron, neutralizing its charge and keeping it from causing harm to the body. Different mechanisms such as free radical scavenging, reduction capacity and metals chelation are often employed to explain the antioxidant potential of a substance or a complex mixture. Here in this study DPPH, ABTS and NO radical scavenging capacity assay are performed to evaluate the antioxidant potential of all the five tested oils.

**i) *DPPH Radical scavenging assay*** : Total free radicals scavenging capacity of the extracts from different plant seed samples were estimated according to the method described by (**Pavithra, K. and Vadivukkarasi, S., 2015**) with slight modification using the stable DPPH radical, which has an absorption maximum at 515nm. A solution of the radical is prepared by dissolving 2.4 mg DPPH in 100 ml methanol. A test solution (5 µl) was added to 3.995 ml of methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 30 mins, probably in a dark place is better. Absorbance of the reaction mixture was measured at 515 nm spectrophotometrically. Absorbance of the DPPH radical without antioxidant i.e blank was also measured. All the experiments were performed in a triplicate manner to minimize any sort of error. The capability to scavenge the DPPH radical was calculated using the following equation

**DPPH scavenging activity (%) = (AB-AA) /AB × 100**

Where, AB is absorbance of blank at t= 0 min

AA is absorbance of the antioxidant at t= 30 mins

A calibration curve plotted with percentage of DPPH scavenged versus concentration of standard antioxidant (Quercetin). (**Rajurkar, N.S. and Hande, S.M., 2011**).

**ii) *ABTS radical scavenging assay* :** Free radical scavenging activity of all the five oils were determined by ABTS radical cation decolorization assay **(Re *et al.*,1999)**. ABTS plus cation radical was produced by the reaction between 7mM ABTS in water and 2.45 mM potassium persulfate (1:1) stored in a dark room at a room temperature for 12-16 hrs before use. ABTS plus solution then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5µl of plant extract to 3.995 ml of diluted ABTS plus solution, the absorbance was measured after 30 mins from the initial mixing. An appropriate solvent of blank was run in

each assay and all the measurements were carried out in a triplicate manner for minimizing the error.

Percent inhibition of absorbance at 734 nm as calculated using the formula,

**ABTS + Scavenging effect (%) = AB-AA/AB × 100**

Where AB is absorbance of ABTS radical + methanol

AA is absorbance of ABTS radical + sample extract / standard

Quercetin was used as standard.

**iii) Nitric oxide scavenging capacity assay :** Nitric oxide radical scavenging capacity was measured spectrophotometrically according to the method described by Jagetia. (**Jagetia *et al., 2004*)** with slight change in the method. A volume of 1ml. sodium nitroprusside in phosphate buffer (0.02M,pH 7.4) was mixed with 1ml of different concentrations (0.02-0.10 mg/ml) of the extracts and standard (ascorbic acid).The reaction mixture was at 25°C for two and a half hours. After that about 1.5 ml of Griess reagent (1% sulphanilamide, 2 % O-phosphoric acid and 0.1 % napthylethylene diamine dihydrochloride ) was added. The mixture was kept for 30 mins and then absorbance was measured at 540 nm against a phosphate buffer blank.

Control was maintained with all chemicals excluding extract. The percentage of scavenging activity of nitric oxide calculated as –

**Scavenging activity (%) = A0 – A1 / A0 × 100**

**Where Ao = absorbance of blank reaction.**

**A1 = absorbance in the presence of sample extract.**

**I. Determination of Totox value :**

The totox value is calculated by the formula combining the values of anisidine value (AV) and peroxide value (PV) that indicates an oils overall oxidation state.The lower the totox value the better is the quality of the oil. The oxidation of all overtime as measured by peroxide value and anisidine value. However detection of peroxide value gives the initial evidence of rancidity in unsaturated fats and oils.

**Peroxide value**-As it is known that peroxide value (PV) is an index that is used to quantify the amount of hydroperoxides present in fats and oils. Generally hydroperoxides are shown to be toxic to humans and it is the primary oil oxidation products formed during the initial stages of oxidation. Although Fourier Transfom Infrared (FTIR) and near Infrared (FT-NIR) spectroscopy methods developed for peroxide value measurement but it has the advantages of analytical speed and automation ,but the instruments are quite expensive and requires extensive calibration. As only low PV doesnot necessarily indicate low level of oxidation, it might be the result of the advanced levels of oil oxidation during which primary oxidation products are converted to secondary oxidation product, lowering the PV but increasing the AV value of the oil. Hence both the PV and AV that is the totox value should be considered for the quality of oil evaluation. **( Nurham Turgut Dunford , 2016).**

Other methods are also available but the peroxide value is the most widely used method that gives the idea to measure the limit to which an oil sample has undergone primary oxidation. The extent of secondary oxidation can be determined from P- anisidine test **(Chakrabarty, M.M., 2003).**

**Determination of Peroxide value –**

Spectrophotometry methods of peroxide values of oils was calculated based on the oxidation of Fe (II) to Fe (III) by hydroperoxides and the formation of the reddish Fe (III) thiocyanate

complex. **(Hornero-Mendez *et al.*, 2001)** .The peroxide value is generally quantified spectrophotometrically.

Here 10 mg of crude oil was dissolved in 50 ml of hexane to obtain a concentration of 2 mg/ml. 200 µl of the 400µg solution was pipetted into the screw capped test tube. It was then dissolved in chloroform of 1ml amount and also acetic acid in the ratio of 2:3 (v/v). 100 µl of Fe (II) solution was added , vortexed for 15 secs and incubated in the dark for 10 mins. After incubation is over, 2ml of deionized water was added to the solution along with 4ml of diethyl ether for the separation of the layer in the solution.The whole solution was then transferred to a separating funnel, where the lower aqueous phase was collected in fresh tubes and the organic phase was discarded. To determine the Fe (III), 1ml of the aqueous phase was mixed with 100 µl of saturated ammonium thiocyanate solution. Keeping in the dark at 10 mins for proper incubation absorbance was measured at 470 nm against a reaction blank (containing all the reagents except the oil sample).

The PV was calculated as –

**Peroxide value = Abs /55.84 ×W× b** [ mEq O2/ kg fat ]

Here w= weight of oil (g)

Abs = absorbance

55.84= atomic weight of Fe3+

b = slope of the Fe (III) calibration curve.

Here Fe (II) stock solution was prepared by gently mixing a solution of 0.4 gm of BaCl2 . 2H20 in 50 ml deionized water with a solution of 0.5 gm of FeSO4.7H20 in 50 ml deionized water. Concentrated HCl of 2ml volume was added to the resulting solution , filtered by Whatman (Grade – 1) filter paper stored in a brown bottle for at least a month.

For Fe (III) calibration,10 mg of FeCl3 is dissolved in 200 ml of 1 % HCL (6ml HCL + 194 ml H2O ) as stock solution. For calibration a set of solutions of increasing Fe (III) concentration in the range of 5-45 μg/ ml was prepared by successive dilutions of the working solution. One ml of each dilution was taken and peroxide value was calculated. The calibration curve was obtained by plotting the absorbance at 470 nm vs Fe (III) concentration.

**p- Anisidine value –** p-Anisidine value is a measure of the secondary oxidation products that are formed by the breakdown of the primary oxidation products during extensive oxidation . The secondary oxidation products are mainly aldehydes such as 2,4-dienals and 2-alkenals.

**Determination of Anisidine value** : The anisidine value (AV) is a measure of the aldehyde levels in an oil or fat in particular those that are unsaturated. To determine the anisidine value (AV) a solution of the oil or fat in isooctane reacts with p-anisidine in glacial acetic acid to form yellowish reaction products. The value is then determined from the absorbance measured at 350 nm, both before and after reaction. As a measure of secondary oxidation products,

Anisidine value is used together with peroxide value to assess the thermally stressed oils .

**(JW Irwin, N. Hedges , 2004 ).**

**Method :**

The oil was dissolved in iso-octane and treated with p-anisidine in acetic acid solution. 0.25% (w/v) anisidine reagent was prepared in acetic acid. The UV_VIS Double Beam Spectrophotometer (**Thermo Fisher, Model No. Genesys. 180**) for this estimation. In the method, 1 g of oil was taken in 25 ml volumetric flask. Iso-octane was added to make up the volume up to 25 ml. The absorbance of the oil solution against pure iso-octane were measured at 350 nm (Ea). 5 ml of each of the oil solution was pipetted into the sample test tubes while 5 ml of the iso-octane was taken as blank. Then anisidine reagent of about 1 ml was added. The

tubes were closed with stoppers, vortexed vigorously and incubated in dark for 10 mins. Finally, absorbance was measured at 350 nm (Eb) in a quartz cuvette.

The anisidine value was measured by using the following formula

**[p- Anisidine value (p-AV) =** $\frac{25 * (1.2 * Eb - Ea)}{W}$**]**

$E_a$ = net absorbance of the fat soln., $E_b$ = net absorbance of the fat + anisidine solution, W = wt. of the sample.

## J. Antimicrobial activity of essential oils :

**Antimicrobial assay using seeded agar diffusion method –** In recent years there has been much interest in research and development for developing new antimicrobial agents from various sources to combat microbial resistance **(Chouhan *et al*., 2017).**

Now a days at present many antibiotics are available for treating various bacterial pathogens, but alongside due to increased multidrug resistance has led to the rising severity of diseases caused by the bacterial pathogens. Bacterial and fungal infections remain the most causative agent that has led to the loss of human lives even today. In addition the use of several antibacterial agents at higher doses causes toxicity in humans.**(Swamy *et al*., review 2016)**. For this reason greater importance has been given to the screening of antimicrobial activity and its evaluation methods. Different types of bioassay methods such as well-diffusion , disk-diffusion and broth or agar dilution are well known and commonly used methods. The lowest concentration of antimicrobial agent that inhibits the growth of the organism in microdilution wells or tubes as observed by the normal eye is called minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC**)** ( **Burt,*et al* 2004**).

The most appropriate bioassays for the determination of MIC value are the dilution methods as these bioassays give the possibility of estimating the concentration of the tested

antimicrobial agent in the agar (agar dilution) or broth medium (macro dilution or microdilution) . Both bactericidal and fungicidal activity of seed oil has been administered.

**Bacterial and Fungal Strains** :

Here all the edible oils (Sunflower, Sterculia, Mustard, Groundnut and Soybean) have been tested to compare the efficiency of Sterculia oil(Java olive oil) against the other edible oils available in the market to detect the antimicrobial properties by applying them at three different concentrations of 1mg/ml,0.5mg/ml and 0.25mg/ml respectively.

Two strains of bacterial test microorganisms used in this study were *Bacillus subtilis* and *Xanthomonas*. Of these gram-positive strain was *Bacillus subtilis* and gram-negative strain was *Xanthomonas. Bacillus subtilis* (MTCC 441), *Xanthomonas*(MTCC 12943).

Two fungal strains were also used for this experiment. They were *Aspergilus niger*(MTCC-9652) and *Penicillium chrysogenum*(MTCC 6795) which were cultured in the laboratory.

**Culture Maintenance and Preparation of Inoculum :**

The isolate of bacteria strains used for the present study were obtained from MTCC (Microbial Type Culture Collection) housed at the Institute of Microbial Technology (IMTECH) at Chandigarh, Punjab,India.The fungal cultures were maintained on Sabouraud Dextrose Agar (SDA) medium and subcultured on Potato Dextrose Agar (PDA)Himedia every 15 days to prevent pleomorphic transformations. Bacterial cultures were grown in nutrient broth (Himedia) at 37°C and maintained on nutrient agar slants at 4°C and cultures were subcultured every 15 days to prevent the pleomorphic transformations.

**Fungicidal Activity of Bromosterculic acid :**

The activity of 5 edible oils against *Penicillium chrysogenum and Aspergilus niger* was assayed by agar well diffusion method. Few fungal spores of the fungal strain was transferred to PDA

(Potato Dextrose Agar Media) slants and was incubated for one week for colony growth. After one week, one loop full of fungal spore of each species was added to the sterile saline water and mixed well. Fungal spore suspension (1 ml) in water was then poured in a sterile Petri dish containing molten PDA and allowed to solidify the plates. A well of 25mm$^2$ size was cut at centre of the petridish and 0.5 ml of seed oil was added with a concentration of 1mg/ml,0.5mg/ml and 0.25mg/ml respectively each. Antibiotic Nystatin was used as a positive control and 0.5ml of DMSO (1%) was used as negative control. The plates were incubated at 28±1°C for 24-48 hrs. The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). All the experiments were performed in triplicate to avoid any form of error . Area of inhibition zone was calculated as:

[Area of inhibition at x ppm = 3.14 ($TR_x^2 – r^2$)]

Where x = concentration used; r = radius, $TR_x$ = Total radius of the inhibition zone at specific concentration **(Biswas *et al.*, 2009)**.

**Bactericidal Activity of Bromosterculic acid:**

Inhibition zone test technique was performed for testing the impact of each seed oil against *Bacillus subtilis* and *Xanthomonas* strain including positive and negative control. Both the strains were cultured on NA (Nutrient Agar Media) slants and incubated for 24 hrs. After 24 h, 1 loop full of bacterial culture was added to sterile nutrient broth with proper mixing and incubated at 37°C for two and half hour. 1ml of bacterial cell suspension was then added to sterile Petri dish containing molten NA media and allowed to solidify. After complete solidification a well of 25mm$^2$ size was cut at centre of the petridish and in this well 0.5 ml of each seed oil with 2000ppm concentration was added. In positive and negative control 0.5ml of antibiotic (ampicillin) and 0.5ml of DMSO (1%) were added respectively. The plates were incubated at 37±1°C for 24 hrs. After incubation, plates were taken out and observations of

inhibition zone were recorded. The experiments were done in triplicate. Area of inhibition zone was calculated using the formula described for fungal effects.

## K. Determination of Iodine value :

Iodine value (IV) is a measure of the total number of double bonds present in fats and oils. It is expressed as the number of grams of iodine that will react with the double bonds in 100 gms of fats or oils **(Thomas *et al*., Ullmann's Encyclopedia of Industrial Chemistry, 2007)**. The determination is conducted by dissolving a weighed sample in a non-polar solvent and then adding glacial acetic acid. The double bonds are reacted with an excess of a solution of iodine monochloride in glacial acetic acid (Wij's solution). Mercuric ions are added to hasten the reaction. After completion of the reaction the excess iodine monochloride is decomposed by iodine by the addition of aqueous potassium iodide solution, which is then titrated with standard sodium thiosulfate solution.

This official method for determination of iodine in vegetable oils or biodiesel is based on titration of the excess of halogen reagent with a sodium thiosulfate solution.This is a time consuming procedure and requires a large amount of Wij's reagent and thus results in a huge wastage. As currently there is an increase in demand in the production of vegetable oils in the market,faster and greener analytical procedures are needed that could give results faster in a controlled manner. For this reason a fast volumetric procedures has been developed for determination of iodine in vegetable oil samples.

**Equipments and accessories :**

A vortex with capacity for up to nine 15 ml Falcon tubes and agitation maximum of 1800 rpm and along with a mixing table.

A centrifuge (model- R-8C Laboratory Centrifuge by REMI) was used for phase separation and the aqueous phases were removed with 1 ml. micropipette (Tarsons). A UV – visible

spectrophotometer ( **Thermo Scientific**) equipped with a 1 cm quartz cuvette was used for the spectrophotometric measurements.

**Reagents and solutions :**

Solutions were prepared using analytical grade chemicals (Merck) and ultrapure water (18.2 M Ω cm). Five different vegetable oils obtained from the seeds of Sterculia, Sunflower, Soybean, Groundnut and Mustard are all extracted and refined under laboratory protocols using n-hexane and it is purified using different other solvents extraction methods. Reference samples of vegetable oil (i.e samples with iodine value previously determined by the reference procedures) were used for the optimization of the procedure and for the preparation of the reference solutions. These vegetable oil samples were diluted with n-hexane ( 1 : 2 ) v/v before analysis.

A 1.2 mmol/L triiodide reference solution was prepared by dissolving 30 mg of $I_2$ in 15 ml of ethanol followed by addition of KI to a final concentration of 0.210 mol/L and marking the volume upto 100 ml in a volumetric flask. Hexane replaced the samples for measurement of the reference signals i.e. those obtained without consumption of iodine for halogenation of the unsaturated compounds.

**Procedure :** The analytical procedure was carried out in a 15 ml Falcon tubes based on the single vial principle.

First, 1.2 ml of vegetable oil samples and 1.2 ml of the $I_3^-$ solution were added to the tube. Then it was shaken for 5 mins in a vortex to extract $I_2$ to the organic phase aiming at reaction with the unsaturated compounds. Now the mixture was centrifuged for 2mins to separate the phases before removal of the aqueous phase with a micropipette. The remaining $I_3^-$ was measured by spectrophotometry at 450 nm. The analytical signal was obtained by the difference between the signals obtained in the absence and presence of the sample. The

procedure was optimized by the univariate method and all the measurements were taken in triplicate manner to remove the percentage of error. **( Samara Soares and Fabio R.P. Rocha 2018).**

## L. Cytotoxicity assay :

Cytotoxicity is one of the most important factor for biological evaluation in invitro studies. Invitro chemicals such as drugs and pesticides has different cytotoxicity mechanisms such as destruction of cell membranes, prevention of protein synthesis and irreversible binding to receptors etc. In order to determine the cell death caused by the destruction of cell membranes, there is an urgency need for cheap , reliable and reproducible short-term cytotoxicity and cell viability assays. Generally cytotoxicity and cell viability assays are based on various cell functions, of which a broad spectrum of cytotoxicity assays are currently used in the fields of toxicology and pharmacology.There are different types of these assays-

i) dye exclusion assays

ii) colorimetric assays

iii) fluorometric assays

iv) luminometric assays

Choosing the most reliable assay is important for obtaining accurate and reliable results.

Viability levels or proliferation rates of cells are good indicators of cell health until the cell health is deteriorated by the effect of physical and chemical agents. These agents cause toxicity on cells via different mechanisms such as destruction of cell-membranes, prevention of protein synthesis, irreversible binding to receptors , inhibition of polydeoxynucleotide elongation and enzymatic reactions. In order to determine the cell death caused by these mechanisms, there is a need for cheap, reliable and reproducible short term cytotoxicity and cell – viability assays.

In-vitro cell viability and cytotoxicity assays with cultured cells are used for cytotoxicity tests of chemicals and for drug screening. These assays are used in oncological research to evaluate both compound toxicity and tumor cell growth ,inhibition during drug development . They are rapid ,inexpensive and do not require the use of animals and thus are useful for testing a large number of samples. Invitro cytotoxicity assays has some advantages such as speed, reduced cost and potential for automation and tests using human cells that is more relevant than some in vivo animal tests. At the end of the experiment the number of alive viable cells compared to how many are dead are found to be justified.

**Colorimetric assays (MTT assay)** –

Principle of colorimetric assays is the measurement of a biochemical marker to evaluate metabolic activity of the cells. Reagents used in colorimetric assays develop a color in response to the viability of cells that allows the colorimetric measurement of cell viability via spectrophotometer. Colorimetric assays are easy to perform and comparatively economical.

MTT assay : MTT [3-(4-5 –dimethyl thiazol- 2- yl) -2-5- diphenyl tetrazolium bromide] assay is one of the most commonly used colorimetric assay to assess cytotoxicity or cell – viability through determination of mitochondrial functions of cells by measuring activity of mitochondrial enzymes such as succinate dehydrogenase . In this assay MTT is reduced to a purple formazan by NADH.

MTT assay method is far superior than other dye exclusion methods because of its easy to use, safe and a high reproducibility and widely used to determine cell viability and cytotoxicity tests. Prior to the measurement of absorbance an organic solvent such as DMSO or isopropanol is required to solubilize the crystals. Additional control experiments should be conducted to reduce false positive or false negative results that are caused by background interference due

to inclusion of particles**. (Ozlem Sultan Aslanturk , 2018; *Genotoxicity- A predictable risk to our Actual World ;*Chapter -1,Google books, )**

**Procedure –** The nontoxic nature of sterculia seed oil and bromosterculic acid can be assumed by measuring their cytotoxic activity on normal cell lines such as (MCF-10A and HB-2) and cancerous cell lines (BT-474, AU-565 and MDA-MB-468) including DMSO as control. By performing the MTT assay using the sterculia oil and bromosterculic acid, it has been obtained that they did not show any cytotoxic activity when applied on normal cell lines.

AU-565, BT-474 and MDA-MB-468 are the major breast cancer cell-lines that are primarily used in breast cancer research. AU-565 cancerous cell line has been obtained from humans (female) and it is linked to the disease involving breast adenocarcinoma that is derived from metastatic site that is the Pleural effusion. BT-474 is another cancerous cell-line that has been obtained from humans too and is linked to the disease involving invasive ductal carcinoma a common type of invasive breast carcinoma that accounts for approximately 70% of breast carcinoma. MDA-MB-468 another cancerous cell line was isolated in 1977 by R.Cailleau, *et al.* obtained from a pleural effusion of a 51 year old black female patient with metastatic adenocarcinoma of the breast. Here both the compound sterculia oil and bromo-sterculic acid was dissolved in DMSO and serially diluted with complete medium to get the range of concentrations of test concentration. DMSO concentration was kept at less than <0.1% in all the samples. Cells maintained in appropriate conditions were sealed in 96 well plates and treated with different concentration of the test samples and incubated at 37°C, 5% CO2 for 96 hours. MTT reagent was added to the wells and incubated for 4 hrs; the dark blue formazan product formed by the cells was dissolved in DMSO under a safety cabinet and the reading was obtained in a spectrophotometer at 550 nm. Percentage inhibitions were calculated and plotted with the concentrations that are used to calculate the IC50 values.

## M. Molecular docking-

Molecular docking study was performed with bromo-sterculic acid [8-(1, 2-dibromo-2-octylcyclopropyl) octanoic acid] showing cytotoxic activity against breast cancer cell line. The possible structural interactions that activate Bax and inhibit P53-Mdm2 complex were studied through Autodock 4.2 (Molecular Graphics Laboratory, The Scripps Research Institute, LaJolla ,USA) software **(Forli *et al*., 2016).** Molecular file of bromo-sterculic acid structure was retrieved from ChemDoc and subsequently optimized and converted to pdb file using open babel. Bax (pdb id: 1F16) and MDM2 (pdb id: 1YCR) were downloaded from Protein Data Bank. The ligand (bromo-sterculic acid) was made flexible to make it adaptable to rigid target (receptor) conformation.Unbounded atoms and water molecules were removed from receptor (Bax/MDM2) by AutoDock tools. Then, hydrogen atoms and charges by Gasteiger computation were assigned to the receptor. Grid box with grid map of dimensions (100 Å × 100 Å × 100 Å for Bax ; 70 Å × 70 Å × 70 Å for MDM2) was placed to cover receptor's surface with grid spacing of 0.375 A **(Adebayo *et al*.,2018).** The rest parameters were set to default. Upon completion of docking, conformation with lowest binding energy was chosen for analysis. Discovery Studio Visualizer 4.1 client, Chimera1.14, and LigPlot were used for post-docking analyses.

## Drug-likeness prediction of bromo-sterculic acid by using Lipinski's rule of "5".

Lipinski's rule of 5 was used to confirm the drug likeness as this rule determines the consistency of orally active drugs **(Lipinski, C. A. (2004)**. In this study the selected compound (bromosterculic acid) was screened by performing the Swiss ADME webtool predictor **(Daina *et al*., 2017).** This webtool predictor provide data on the numbers of hydrogen acceptors, hydrogen donors and rotatable bonds.

## N. Statistical Analysis :

Significant differences among the fatty acids composition of all the five oils namely Sterculia, sunflower, groundnut , mustard and soybean were evaluated by Z test analysis. Values obtained from biochemical tests and antioxidant assays were analyzed using independent sample T-test and the significance level was set at p <0.05.All the experiments were conducted three times for perfection and the data was represented as mean values and standard deviation of the same. SPSS Statistics 19 software for windows had been used for all the statistical analysis (SPSS 2009). Here also the student t-test based on the R software that depends on our experimental datasets has been performed for the experiments(mainly bacterial and fungal samples) that include the datasets and has been conducted three times to assess the quality and viability of the result.

Here we have perform the pairwise hypopthesis tests based on the alkaline earth and heavy metals in between any two oil products.For the sake of convenience we consider $O(m)_i$ $and$ $O(n)_i$ for the testing process. The notation $O(m)$ denotes the $m$-th Oil product and $O(m)_i$ indicates the $i$th metal present in the $m$-th Oil proudct. The pairwise tests are conducted for each and every earth and heavy metal elements for the separate oil products. The statistical test was performed between these paired sets of elements to draw any statistical inference whether the amount are significantly different or not. This testing of hypothesis can be formulated by considering the null hypothesis as

$$H_0: (O(m)_i = O(n)_i)$$

against the alternative hypothesis

$$H_1: (O(m)_i > O(n)_i)$$

The estimated values are obtained from the non-linear least square regression. The underlying assumption behind the consistency of the regression procedure is that the errors $\in_i$ are normally

distributed. It implies that the estimated model parameters i.e. $\hat{\theta}$ can also be treated as the maximum likelihood estimator (MLE) (**Seber and Wild 2003)**. Now, from the asymptotic theory of MLE we can say that the distribution of the model parameters $\widehat{\theta_i} \sim N\left(\theta_i, var\left(\widehat{\theta_i}\right)\right)$ for $i = 1(1)4$. Here the $var\left(\widehat{\theta_i}\right)$ represents the asymptotic variance of the corresponding parameters. The aforementioned discussion helps us to construct the following test statistic in order to pursue the testing process

$$\tau_{mn} = \frac{O(m)_i - O(n)_i}{\sqrt{var(O(m)_i) + var(O(n)_i)}}$$

Here, the null distribution (the distribution of the test statistic when the null hypothesis is true) of the test statistic follows the standard normal distribution. Now, the rejection of the null hypothesis occurs at some critical value $\tau_{mn} > Z_\alpha$, where $Z_\alpha$ represents the $100(1-\alpha)$ percentile of the standard normal distribution.

# Chapter-4

# Results

**A) LC-MS analysis of newly synthesized bromo compound -**

LC-MS spectra of purified hexane dissolved bromosterculic acid (BSA) showed only one sharp peak at retention time (Rt) 26.762 mins. as shown in Fig: 7(a) that corresponded to the chemical compound Bromosterculic acid with mass of 453.2062 g/mol Fig:7(b). From the molecular wt, it is evident that two bromine atoms were attached to the cyclopropene ring of sterculic acid and the IUPAC name of the newly synthesized bromo compound is 8-(1,2-dibromo-2-octylcyclopropyl) acid or bromosterculic acid.

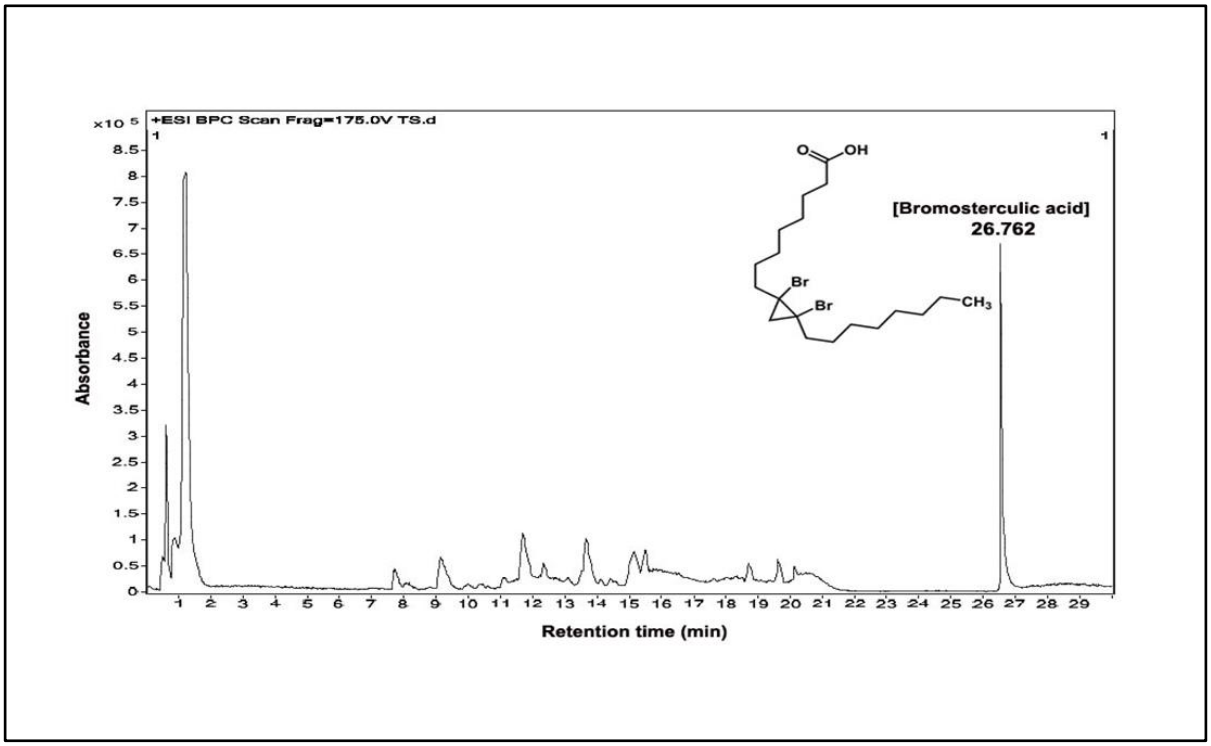



**Fig:7(a) LC-MS analysis of the bromo-sterculic acid**

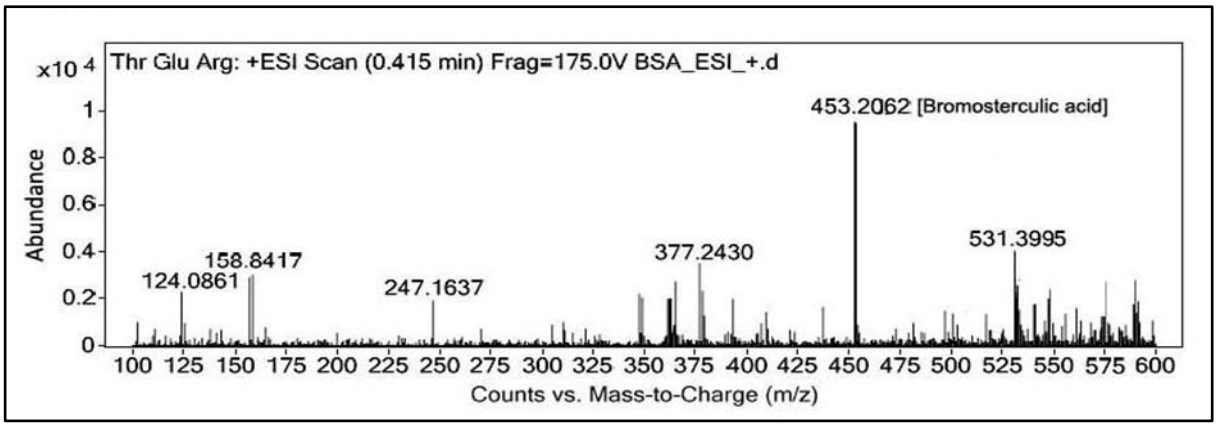



**Fig.7(b) Counts vs Mass to Charge (m/z)**

## B) Proximate Composition of Seeds :

**Proximate composition :** Significant variations were obtained among the five oilseed samples with respect to the crude oil content. Maximum amount of crude oil obtained was from the seeds of Sterculia (nearly 58%) and least was obtained from Soybean (16.3%), Sunflower, Groundnut and Mustard contains 47.5%, 49.8% and 31.4% respectively **( Chakravarty S, Dhingra and K.K. Singh, 2003) .** Like the oil content the crude protein content of the five oil seed samples were also variable. Significant variations was observed among the five oil seed samples. Sterculia seeds contain nearly 38.43gm of protein, followed by groundnut which contains 38.61gm and soybean contains 37.69gm. Sunflower contains 42gm and mustard contains the least 28gms of protein respectively(**Wang *et al.*, 2016** ).

In case of lipid content it was found that highest lipid content was found in Sterculia (58.4 gm/100 gm) followed by the sunflower which contains nearly about 51 gm. Groundnut contains about 50 gm and mustard contains about 36 gm. Least amount of lipid has been obtained from soybean which contains about 18 gm. **( BLIGH and Dyer, 1959 ).**

In comparison to other oilseeds present it has been observed that soybean contains highest amount of soluble sugar about 16.24 gm per 100 gm. **( Hedge,JE and Hofreiter, BT (1962) In : Carbohydrate Chemistry 17)** .

The moisture content was found to be variable among the five seed samples. Soybean oilseeds consist of maximum amount of moisture of about 13.5 gm per 100 gm, while sterculia consist of least moisture of 5.28 gm/100 gm. The other three oilseeds consist of respectively Sunflower (7.18 gm), Groundnut (5.98 gm) and Mustard consist of (9.12 gm) respectively. **[AOAC, 1980,Official methods of Analysis,(13th Edition)].** All the results were concluded after the experiment was performed three times to minimize the percentage of error.

**Table 1. Proximate compositions of *Sterculia foetida* seeds along with sunflower, groundnut, mustard and soybean seeds.**

| | Sterculia | Sunflower | Groundnut | Mustard | Soybean |
|---|---|---|---|---|---|
| **NUTRITIONAL COMPONENTS (g/100g)** | | | | | |
| **Oil Content** | 58.7± 2.37[a] | 47.5± 2.22[a] | 49.8± 1.62[a] | 31.4± 1.45[b] | 16.3± 1.19[c] |
| **Lipid Content** | 58 .4± 4.52[a] | 51± 3.50[a] | 50± 6.52[a] | 36± 3.36[a] | 18.39± 4.52[b] |
| **Protein Content** | 38.43± 2.81[a] | 20.91± 3.08[c] | 38.61± 3.02[a] | 28.8± 4.05[b] | 37.69± 2.66[a] |
| **Soluble Sugar Content** | 1.73±1.34[c] | 2.54± 1.22[c] | 1.46± 1.26[c] | 6.74±1.05[b] | 16.24±2.73[a] |
| **Moisture Content** | 5.28± 3.22[b] | 7.18± 3.22[b] | 5.98± 2.01[b] | 9.12± 4.33[a] | 13.5± 2.44[a] |

## C). Mineral composition of seed flour :

After performing heavy metal analysis using Atomic Emission Spectroscopy (AES**) (Agilent Technologies )** the presence of Alkaline earth metals (Na , K, Ca, Mg) and heavy metals (Cu, Mn , Pb , Fe, Zn ) are being detected to determine the amount of which heavy metal is present in the least amount and which in high amount among the seed flour of Sunflower , Sterculia , Groundnut , Mustard and Soybean respectively. Among the heavy metals, Sterculia seeds contain lowest amount of copper (16.68 mg/kg), lead (12.7 mg/kg), manganese (10.7 mg/kg) while iron (938.68 mg/kg) is present in good amount. Other than these Zinc (138.33 mg/kg)

are the main metals in Sterculia as compared to other tested seeds. Highest amount of lead (48.67 mg/kg) , iron (146.34 mg/kg) were detected in mustard seeds where as highest amount of copper (52.68 mg/kg) and manganese (62.3 mg/ kg) were recorded from groundnut seeds. Among the alkaline earth metals only sodium (350.67 mg/kg) ,magnesium (3436 mg/kg) and potassium (19858 mg/kg) was found in highest quantity in the seeds of sterculia compared to other oilseeds as shown in the table-2. Only calcium (5218.33 mg/kg) is present in highest quantity in the seeds of mustard but less in other oilseeds.

**Table-2. Mineral composition of seed flours of Sterculia along with Sunflower, Groundnut, Mustard and Soybean.**

| | **Sterculia** | **Sunflower** | **Groundnut** | **Mustard** | **Soybean** |
|---|---|---|---|---|---|
| **ALKALINE EARTH METALS (mg/kg)** | | | | | |
| Magnesium | 590.33±4.51$^{b}$ | 3251±3.61$^{a}$ | 3436±6.55$^{a}$ | 3040±72.11$^{a}$ | 2756.67±40.5$^{a}$ |
| Sodium | 350.67±24.44$^{a}$ | 89.33±3.05$^{b}$ | 335±4.35$^{a}$ | 52.67±6.42$^{b}$ | 181±3.60$^{a}$ |
| Potassium | 5293.33±345.5$^{b}$ | 6455.67±6.0$^{b}$ | 14500±2.01$^{a}$ | 6882±65.21$^{b}$ | 19858±15.62$^{a}$ |
| Calcium | 590.33±4.51$^{c}$ | 3251±3.61$^{a}$ | 2002.33±5.8$^{b}$ | 5218.33±7.6$^{a}$ | 3124±5.29$^{a}$ |
| **HEAVY METALS (mg/kg)** | | | | | |
| Copper | 16.67± 4.16$^{b}$ | 20±2.03$^{b}$ | 52.67±2.08$^{a}$ | 38.67±1.15$^{a}$ | 48±3.0$^{a}$ |
| Lead | 12.6± 3.05$^{b}$ | 10.33±1.52$^{b}$ | 44±2.35$^{a}$ | 48.67± 8.14$^{a}$ | 38.33±5.50$^{a}$ |
| Manganese | 10.6± 1.15$^{b}$ | 21±2.01$^{b}$ | 62±2.64$^{a}$ | 41.33±2.5$^{a}$ | 41±1.03$^{a}$ |
| Iron | 38.67± 2.51$^{b}$ | 53.33±1.52$^{b}$ | 111±2.64$^{a}$ | 146.33±1.52$^{a}$ | 38.67±2.51$^{b}$ |
| Zinc | 138.33±2.08$^{a}$ | 52.67±3.05$^{b}$ | 75±5.56$^{a}$ | 38.67±1.15$^{b}$ | 52.67±1.52$^{b}$ |

*Mean values ± standard deviation for $n = 3$*

**Values (means± SD) with different index letters are statistically significantly different ($P < 0.05$).*

**Table-3: Testing of hypothesis in between the heavy metals present in the *Sterculia* and Groundnut**

| Heavy metal | Levels | Null and Alternative Hypothesis | Test Statistic | p-value | Decision at 5% level |
|---|---|---|---|---|---|
| **Copper** | Comparison of the presence of Copper among the *Sterculia* and Groundnut | $H_0: Gn_{Cu} = St_{Cu}$ against $H_1: Gn_{Cu} > St_{Cu}$ | 10.938 | 0.0002 | $\boldsymbol{H_0}$ rejected |
| **Lead** | Comparison of the presence of Lead among the *Sterculia* and Groundnut | $H_0: Gn_{pb} = St_{pb}$ against $H_1: Gn_{pb} > St_{pb}$ | 12.135 | 0.0001 | $\boldsymbol{H_0}$ rejected |
| **Manganese** | Comparison of the presence of Manganese among the *Sterculia* and Groundnut | $H_0: Gn_{mn} = St_{mn}$ against $H_1: Gn_{mn} > St_{mn}$ | 25.14 | $7.422 \times 10^{-6}$ | $\boldsymbol{H_0}$ rejected |
| **Iron** | Comparison of the presence of Iron among the *Sterculia* and Groundnut | $H_0: Gn_{fe} = St_{fe}$ against $H_1: Gn_{fe} > St_{fe}$ | 28.01 | $4.829 \times 10^{-6}$ | $\boldsymbol{H_0}$ rejected |
| **Zinc** | Comparison of the presence of Zinc among the *Sterculia* and Groundnut | $H_0: Gn_{zn} = St_{zn}$ against $H_1: Gn_{zn} > St_{zn}$ | 15.06 | $5.652 \times 10^{-6}$ | $\boldsymbol{H_0}$ rejected |

**Table-4: Testing of hypothesis in between the alkaline earth metals present in the *Sterculia* and Groundnut.**

| Earth metal | Levels | Null and Alternative Hypothesis | Test Statistic | p-value | Decision at 5% level |
|---|---|---|---|---|---|
| **Magnesium** | Comparison of the presence of Magnesium among the *Sterculia* and Groundnut | $H_0: Gn_{mg} = St_{mg}$ against $H_1: Gn_{mg} > St_{mg}$ | 505.58 | $4.587 \times 10^{-11}$ | $\boldsymbol{H_0}$ rejected |
| **Sodium** | Comparison of the presence of Sodium among the *Sterculia* and Groundnut | $H_0: Gn_{na} = St_{na}$ against $H_1: Gn_{na} > St_{na}$ | 0.8924 | 0.211 | $\boldsymbol{H_0}$ accepted |
| **Calcium** | Comparison of the presence of Calcium among the *Sterculia* and Groundnut | $H_0: Gn_{ca} = St_{ca}$ against $H_1: Gn_{ca} > St_{ca}$ | 13.092 | $9.825 \times 10^{-5}$ | $\boldsymbol{H_0}$ rejected |
| **Potassium** | Comparison of the presence of Potassium among the *Sterculia* and Groundnut | $H_0: Gn_k = St_k$ against $H_1: Gn_k > St_k$ | 21.82 | $1.303 \times 10^{-5}$ | $\boldsymbol{H_0}$ rejected |

**Table-5: Testing of hypothesis in between the heavy metals present in the *Sterculia* and Sunflower**.

| Heavy metal | Levels | Null and Alternative Hypothesis | Test Statistic | p-value | Decision at 5% level |
|---|---|---|---|---|---|
| **Copper** | Comparison of the presence of Copper among the *Sterculia* and Sunflower | $H_0: Sf_{Cu} = St_{Cu}$ against $H_1: Sf_{Cu} > St_{Cu}$ | 1.02 | 0.18 | $\boldsymbol{H_0}$ accepted |
| **Lead** | Comparison of the presence of Lead among the *Sterculia* and Sunflower | $H_0: Sf_{pb} = St_{pb}$ against $H_1: Sf_{pb} > St_{pb}$ | 0.966 | 0.194 | $\boldsymbol{H_0}$ accepted |
| **Manganese** | Comparison of the presence of Manganese among the *Sterculia* and Sunflower | $H_0: Sf_{mn} = St_{mn}$ against $H_1: Sf_{mn} > St_{mn}$ | 6.327 | 0.001 | $\boldsymbol{H_0}$ rejected |
| **Iron** | Comparison of the presence of Iron among the *Sterculia* and Sunflower | $H_0: Sf_{fe} = St_{fe}$ against $H_1: Sf_{fe} > St_{fe}$ | 7.045 | 0.001 | $\boldsymbol{H_0}$ rejected |
| **Zinc** | Comparison of the presence of Zinc among the *Sterculia* and Sunflower | $H_0: Sf_{zn} = St_{zn}$ against $H_1: Sf_{zn} > St_{zn}$ | 32.77 | $2.584 \times 10^{-6}$ | $\boldsymbol{H_0}$ rejected |

**Table-6: Testing of hypothesis in between the alkaline earth metals present in the *Sterculia* and Sunflower**

| Earth metal | Levels | Null and Alternative Hypothesis | Test Statistic | p-value | Decision at 5% level |
|---|---|---|---|---|---|
| **Magnesium** | Comparison of the presence of Magnesium among the *Sterculia* and Sunflower | $H_0: Sf_{mg} = St_{mg}$ against $H_1: Sf_{mg} > St_{mg}$ | 651.72 | $1.662 \times 10^{-11}$ | $\boldsymbol{H_0}$ rejected |
| **Sodium** | Comparison of the presence of Sodium among the *Sterculia* and Sunflower | $H_0: Sf_{na} = St_{na}$ against $H_1: Sf_{na} > St_{na}$ | 15.004 | $5.74 \times 10^{-5}$ | $\boldsymbol{H_0}$ rejected |
| **Calcium** | Comparison of the presence of Calcium among the *Sterculia* and Sunflower | $H_0: Sf_{ca} = St_{ca}$ against $H_1: Sf_{ca} > St_{ca}$ | 3.422 | $8.908 \times 10^{-6}$ | $\boldsymbol{H_0}$ rejected |
| **Potassium** | Comparison of the presence of Potassium among the *Sterculia* and Sunflower | $H_0: Sf_k = St_k$ against $H_1: Sf_k > St_k$ | 24.02 | 0.0133 | $\boldsymbol{H_0}$ rejected |

**Table-7: Testing of hypothesis in between the heavy metals present in the Sterculia and Mustard**

| Heavy metal | Levels | Null and Alternative Hypothesis | Test Statistic | p-value | Decision at 5% level |
|---|---|---|---|---|---|
| **Copper** | Comparison of the presence of Copper among the *Sterculia* and Mustard | $H_0: Ms_{Cu} = St_{Cu}$ against $H_1: Ms_{Cu} > St_{Cu}$ | 7.201 | 0.0009 | $\boldsymbol{H_0}$ rejected |
| **Lead** | Comparison of the presence of Lead among the *Sterculia* and Mustard | $H_0: Ms_{pb} = St_{pb}$ against $H_1: Ms_{pb} > St_{pb}$ | 5.85 | 0.0021 | $\boldsymbol{H_0}$ rejected |
| **Manganese** | Comparison of the presence of Manganese among the *Sterculia* and Mustard | $H_0: Ms_{mn} = St_{mn}$ against $H_1: Ms_{mn} > St_{mn}$ | 15.663 | $4.85 \times 10^{-5}$ | $\boldsymbol{H_0}$ rejected |
| **Iron** | Comparison of the presence of Iron among the *Sterculia* and Mustard | $H_0: Ms_{fe} = St_{fe}$ against $H_1: Ms_{fe} > St_{fe}$ | 51.721 | $4.181 \times 10^{-7}$ | $\boldsymbol{H_0}$ rejected |
| **Zinc** | Comparison of the presence of Zinc among the *Sterculia* and Mustard | $H_0: Ms_{zn} = St_{zn}$ against $H_1: Ms_{zn} > St_{zn}$ | 34.046 | $2.21 \times 10^{-6}$ | $\boldsymbol{H_0}$ rejected |

**Table-8: Testing of hypothesis in between the alkaline earth metals present in the Sterculia and Mustard**

| Earth metal | Levels | Null and Alternative Hypothesis | Test Statistic | p-value | Decision at 5% level |
|---|---|---|---|---|---|
| **Magnesium** | Comparison of the presence of Magnesium among the *Sterculia* and Mustard | $H_0: Ms_{mg} = St_{mg}$ against $H_1: Ms_{mg} > St_{mg}$ | 47.95 | $5.66 \times 10^{-7}$ | $\boldsymbol{H_0}$ rejected |
| **Sodium** | Comparison of the presence of Sodium among the *Sterculia* and Mustard | $H_0: Ms_{na} = St_{na}$ against $H_1: Ms_{na} > St_{na}$ | 16.67 | $3.79 \times 10^{-5}$ | $\boldsymbol{H_0}$ rejected |
| **Calcium** | Comparison of the presence of Calcium among the *Sterculia* and Mustard | $H_0: Ms_{ca} = St_{ca}$ against $H_1: Ms_{ca} > St_{ca}$ | 4.373 | $4.98 \times 10^{-5}$ | $\boldsymbol{H_0}$ rejected |
| **Potassium** | Comparison of the presence of Potassium among the *Sterculia* and Mustard | $H_0: Ms_k = St_k$ against $H_1: Ms_k > St_k$ | 15.56 | 0.006 | $\boldsymbol{H_0}$ rejected |

**Table-9: Testing of hypothesis in between the heavy metals present in the *Sterculia* and Soybean**

| Heavy metal | Levels | Null and Alternative Hypothesis | Test Statistic | p-value | Decision at 5% level |
|---|---|---|---|---|---|
| **Copper** | Comparison of the presence of Copper among the *Sterculia* and Soybean | $H_0: Soy_{Cu} = St_{Cu}$ against $H_1: Soy_{Cu} > St_{Cu}$ | 8.635 | 0.0004 | $\boldsymbol{H_0}$ rejected |
| **Lead** | Comparison of the presence of Lead among the *Sterculia* and Soybean | $H_0: Soy_{pb} = St_{pb}$ against $H_1: Soy_{pb} > St_{pb}$ | 5.763 | 0.0022 | $\boldsymbol{H_0}$ rejected |
| **Manganese** | Comparison of the presence of Manganese among the *Sterculia* and Soybean | $H_0: Soy_{mn} = St_{mn}$ against $H_1: Soy_{mn} > St_{mn}$ | 28.08 | $4.782 \times 10^{-5}$ | $\boldsymbol{H_0}$ rejected |
| **Iron** | Comparison of the presence of Iron among the *Sterculia* and Soybean | $H_0: Soy_{fe} = St_{fe}$ against $H_1: Soy_{fe} > St_{fe}$ | 0 | 0.5 | $\boldsymbol{H_0}$ rejected |
| **Zinc** | Comparison of the presence of Zinc among the *Sterculia* and Soybean | $H_0: Soy_{zn} = St_{zn}$ against $H_1: Soy_{zn} > St_{zn}$ | 46.921 | $6.17 \times 10^{-7}$ | $\boldsymbol{H_0}$ rejected |

**Table-10: Testing of hypothesis in between the alkaline earth metals present in the *Sterculia* and Soybean.**

| Earth metal | Levels | Null and Alternative Hypothesis | Test Statistic | p-value | Decision at 5% level |
|---|---|---|---|---|---|
| **Magnesium** | Comparison of the presence of Magnesium among the *Sterculia* and Soybean | $H_0: Soy_{mg} = St_{mg}$ against $H_1: Soy_{mg} > St_{mg}$ | 75.338 | $9.301 \times 10^{-8}$ | $\boldsymbol{H_0}$ rejected |
| **Sodium** | Comparison of the presence of Sodium among the *Sterculia* and Soybean | $H_0: Soy_{na} = St_{na}$ against $H_1: Soy_{na} > St_{na}$ | 9.712 | 0.0003 | $\boldsymbol{H_0}$ rejected |
| **Calcium** | Comparison of the presence of Calcium among the *Sterculia* and Soybean | $H_0: Soy_{ca} = St_{ca}$ against $H_1: Soy_{ca} > St_{ca}$ | 34.077 | 0.018 | $\boldsymbol{H_0}$ rejected |
| **Potassium** | Comparison of the presence of Potassium among the *Sterculia* and Soybean | $H_0: Soy_k = St_k$ against $H_1: Soy_k > St_k$ | 3.095 | $2.21 \times 10^{-6}$ | $\boldsymbol{H_0}$ rejected |

**D). Comparative analysis of the fatty acid profiling with the respective retention time :**

GCMS Analysis of the FAME samples of all the five oils showed that some fatty acids like Palmitic acid, Arachidic acid, behenic acid and lignoceric acid are common in all the five types of oil. Only a special type of fatty acid named Sterculic acid is present only in Sterculia seed oil. All the fatty acids were analysed after saponication with KOH and precipitation of fatty acids by adding dilute HCl (5% w/w)

**Table-11**

**Comparative fatty acid profiling with respective retention times based on GC-MS analysis of five seed oils.**

| RT | Name of Fatty acid | Type of Fatty acid | No. of carbon atom | Sterculia | Sunflower | Groundnut | Mustard | Soybean |
|---|---|---|---|---|---|---|---|---|
| 12.098 | Caprylic acid | SFA | $C_8H_{16}O_2$ | | + | + | | |
| 14.892 | Pelargonic acid | SFA | C9H18O2 | + | | | | |
| 18.205 | 8-Nonyonic acid | MUFA | C9H16O2 | + | | | | |
| 20.473 | 9-oxononanoic acid | MUFA | C9H16O3 | | | + | | |
| 20.488 | 9-oxocapric acid | MUFA | C9H16O3 | | + | | | |
| 22.548 | 9-oxodecanoic acid | MUFA | C10H18O3 | + | | | | |
| 23.212 | 2-methyl-azelaic acid | MUFA | C10H18O4 | + | | | | |
| 27.218 | Myristic acid | SFA | C14H28O2 | + | + | + | | + |
| 30.933 | Palmitoleic acid | MUFA | C16H30O2 | + | + | + | + | + |
| 31.472 | Palmitic acid | SFA | C16H32O2 | | + | + | + | + |
| 31.478 | Methyl 8-(2-hexylcyctopropyl)octanoate | CFA | C18H34O2 | + | | + | | |
| 33.231 | Margaric acid | SFA | C17H34O2 | + | | + | | + |
| 33.486 | 8-octadecynoic acid | MUFA | C18H32O2 | + | | | | |
| 34.643 | Linoleic acid | PUFA | C18H32O2 | + | + | + | + | + |
| 34.783 | Oleic acid | MUFA | C18H34O2 | + | + | + | | + |
| 35.338 | Stearolic acid | MUFA | C18H32O2 | + | | | | |
| 35.738 | Stearic acid | SFA | C18H36O2 | + | + | + | + | + |
| 36.501 | 10-Nonadecenoic acid | MUFA | C19H36O2 | | | + | | |
| 36.604 | Sterculic acid | CFA | C19H36O2 | + | | | | |
| 37.642 | 7,10-Octadecadienoic acid | PUFA | C19H34O2 | | | | | + |
| 37.756 | alpha-Linolenic acid | PUFA | C18H30O2 | | | + | + | + |
| 37.777 | Stearolic acid | MUFA | C18H32O2 | | + | | | |
| 38.047 | Methyl cis-3-octyloxiraneoctanoate | MUFA | C19H36O3 | | | + | | |
| 38.093 | 11,13-Eicosadienoic acid | PUFA | C20H36O2 | | | | + | |
| 38.161 | cis-9,10-Ethoxystearic acid | CFA | C18H34O3 | | + | | | |
| 38.192 | 10-Oxooctadecanoic acid | MUFA | C18H34O3 | + | | | | |
| 38.254 | Gondoic acid | MUFA | C20H38O2 | | + | + | + | + |
| 38.705 | Arachidic acid | SFA | C20H40O2 | + | + | + | + | + |
| 38.747 | Gamma-Linolenic acid | PUFA | C19H32O2 | | + | | | |
| 39.982 | Cyclopropanedodecanoic acid | CFA | C24H46O2 | + | | | | |
| 40.013 | Lactobacillic acid | CFA | C19H36O2 | | | | | |
| 40.288 | Heneicosylic acid | SFA | C21H42O2 | | | + | | |
| 41.191 | cis-Linoleic acid | PUFA | C18H32O2 | | + | | | |
| 42.016 | Erucic acid | MUFA | C22H42O2 | | | | + | |
| 42.529 | Behenic acid | SFA | C22H44O2 | + | + | + | + | + |
| 44.325 | Tricosylic acid | SFA | C23H46O2 | | + | + | | |
| 45.731 | Nervonic acid | MUFA | C24H46O2 | | | | + | |
| 46.001 | Lignoceric acid | SFA | C24H48O2 | + | + | + | + | + |
| 48.657 | Cerotic acid | SFA | C26H52O2 | | + | + | | |

**SFA- Saturated fatty acid, MUFA- Monounsaturated fatty acid, PUFA- polyunsaturated fattyacid, CFA- cyclopropenoid fatty acid.'+' – presence of fatty acid, Blank- absence.**

From the above table-11, using the Gas-Chromatography and Mass spectrophotometry(GC-MS) method a total of 5 vegetable oil samples collected after performing extraction in the laboratory were used for the analysis of fatty acid composition .Types and number of samples collected were the Sunflower oil , Groundnut , Soybean and Mustard respectively. The content of following saturated and unsaturated fatty acids was tested in samples those are Caprylic acid (C8 : 0), pelargonic acid (C9 : 0) , 8 – Nonyonic acid (C9 : 1) , 9- Oxononanoic acid ( C9 : 1) , 9 oxodecanoic acid (C 10 : 1), 2- Methyl-azelaic acid ( C 10 : 1) , Myristic acid ( C14 : 0), Palmitoleic acid (C16 : 1) , Palmitic acid ( C16 : 0) , Methyl 8 – (2- hexylcyclopropyl) octanoate C18, Margaric acid (C17 : 0) , 8 octadecynoic acid (C18 : 0), Linoleic acid (C18 : 2), Oleic acid (C18 :1), Stearolic acid (C18 : 1), Stearic acid (C18 : 0), 10- Nonadecenoic acid (C19 : 1), Sterculic acid (C19), 7,10 – Octadecadienoic acid (C19 : 2), Alpha- Linolenic acid (C18 : 3), Stearolic acid (C18 : 1), Cis-9,10 – Ethoxystearic acid (C18), Methyl cis -3-octyloxiraneoctanoate (C19 : 1), 11,13 – Eicosadienoic acid, cis- 9,10- Ethoxystearic acid (C18), 10- Oxooctadecanoic acid (C18 : 1), Gondoic acid (C20 : 1), Arachidic acid (C20 : 0) ,Gamma- Linolenic acid (C19 : 3), Cyclopropanedodecanoic acid (C24), Lactobacillic acid (C19), Heneicosylic acid (C21 : 0), cis- Linoleic acid (C18 :2), Erucic acid (C22 :1), Behenic acid (C22: 0), Tricosylic acid (C23 : 0), Nervonic acid (C24 : 1), Lignoceric acid (C24 : 0),Cerotic acid (C26 : 0). The GCMS chromatogram of FAME samples of all the five oils shows their fatty acid composition in the table provided. In sterculia oil there were total 19 fatty acids consisting of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and cyclopropenoid fatty acids (CFA).These fatty acids are present in the ratios of 7:8:1:3. In sterculia oil, the carbon number in the fatty acids varies from C9 to C24. In sunflower oil, the fatty acid composition (SFA : MUFA : PUFA : CFA) was in the ratio of 9:5:3:1, whereas in groundnut oil, mustard and soybean oil it was 12:6:3:2 , 5:4:3:0 and 7:3:3:1 respectively. Incase of sterculia , sunflower and groundnut oil short chain

fatty acids i.e C8 to C10 were present only. Other fatty acids like palmitoleic acid , Linoleic acid , Stearic acid, Arachidic acid, Behenic acid , Lignoceric acid were in common in all the five oils, while each of them had unique fatty acids.

**E). Analyses of fatty acid composition :**

**Table-12.**

**Fatty acid composition of all the five seed oils namely Sterculia, Sunflower, Groundnut, Mustard and Soybean based on MDLC analyses of the FAME samples of respective oils**.

| Name of the fatty acid | Fatty acid content (mg/ml) | | | | |
|---|---|---|---|---|---|
| | **Sterculia** | **Sunflower** | **Mustard** | **Groundnut** | **Soybean** |
| **Sterculic acid (C 19:1) CFA** | 0.55± 0.01 | 0 | 0 | 0 | 0 |
| **Linolenic acid (C 18:3) PUFA** | 1.12± 0.01 | 0.52±0.01 | 0.76±0.00 | 1.25±0.05 | 0.62± 0.02 |
| **Linoleic acid (C18:2) PUFA** | 0.32±0.040 | 0.04±0.003 | 0.12±0.039 | 0 | 0.1± 0.02 |
| **Palmitic acid (C16:0) SFA** | 1.23± 0.16 | 0.20± 0.04 | 0.76±0.032 | 0.04±0.002 | 0.76± 0.029 |
| **Myristic acid (C14:0) SFA** | 0.06± 0.001 | 0.033± 0.001 | 0.015±0.002 | 0.04± 0.001 | 0.012± 0.009 |
| **Oleic Acid (C18:1) MUFA** | 0.016± 0.006 | 0.62± 0.13 | 0.18± 0.019 | 0.024± 0.011 | 0.142± 0.00 |
| **Total saturated fatty acids** | 1.29± 0.015 | 0.233± 0.025 | 0.775± 0.017 | 0.08± 0.012 | 0.772± 0.019 |
| **Total monounsaturated fatty acids** | 0.016± 0.006 | 0.62± 0.013 | 0.18± 0.019 | 0.024± 0.011 | 0.142± 0.00 |
| **Total polyunsaturated fatty acids** | 1.44± 0.020 | 0.56± 0.016 | 0.88± 0.019 | 1.25± 0.005 | 0.72± 0.015 |
| **Total cyclopropenoid fatty acids** | 0.55± 0.001 | 0 | 0 | 0 | 0 |
| **TOTAL** | **3.296± 0.013** | **1.413± 0.019** | **1.835± 0.018** | **1.354± 0.072** | **1.634± 0.023** |

From the above table-12 it can be concluded that the MDLC analysis of FAME samples of all the five tested oils shows the fatty acid composition has been shown.

Highest amount of polyunsaturated fatty acids(PUFA) are found in sterculia seed oil (1.44mg/ml) followed by groundnut (1.25 mg/ml) and least amount in sunflower oil (0.56mg/ml). Total monounsaturated fatty acids (MUFA) content is also high in sunflower oil (0.62mg/ml), followed by mustard (0.18mg/ml)and least in sterculia (0.016 mg/ml).Total saturated fatty acids (SFA) is also high in sterculia (1.29 mg/ml) and least in groundnut (0.08mg/ml).Only the cyclopropenoid fatty acids(CFA) is found to be present in sterculia oil but absent in other oils. Thus the total fatty acids present is found to be highest in sterculia oil and least in groundnut oil. In the five oils , the 6 fatty acids presents are linolenic aids(C18:3), linoleic acids(C18:2) ,palmitic acid (C16:0),myristic acid (C14:0), oleic acid (C18:1) and sterculic acid (C19:1).Of these the linolenic and linoleic acids are the polyunsaturated fatty acids (PUFA) ,palmitic and myristic acid are the saturated fatty acids (SFA) and oleic acid is the monounsaturated fatty acids (MUFA) and only sterculic acid is the cyclopropenoid fatty acids (CFA). In the five oils, the carbon number in the fatty acids varies from C14 to C19 .Total SFA was present higher in sterculia oil (1.29 mg/ml) and lowest in groundnut oil (0.08mg/ml).Other than that rest other oils i.e sunflower oil, mustard oil and soybean oil consists of 0.23mg/ml, 0.77mg/ml and 0.77 mg/ml respectively. Total MUFA was present higher in sunflower oil (0.62mg/ml) and lowest in sterculia oil (0.016mg/ml) and mustard, soybean and groundnut oil consists of 0.18mg/ml, 0.14mg/ml and 0.02 mg/ml respectively. Finally total PUFA were present in higher quantity in sterculia oil (1.44 mg/ml) and lowest in sunflower oil (0.56 mg/ml). The remaining mustard , groundnut and soybean oil consists of (0.88mg/ml),(1.25mg/ml) and (0.72 mg/ml) fatty acids respectively. Calculating the total fatty acids it shows that sterculia oil consists of highest amount of fatty acids i.e 3.296 mg/ml and groundnut oil consists the lowest 1.35 mg/ml.

**F. Determination of Totox value and Iodine value :**

Oxidation of oil depends on the totox value which is calculated by the formula AV + 2PV to indicate the oils overall oxidation state. The lower the TOTOX value the better the quality of oil.

Highest para- anisidine value was obtained by groundnut oil (5.45) followed by mustard oil (5.09) where as lowest para- anisidine value was detected in sunflower oil (2.99). Sterculia and Soybean oil showed moderate para anisidine value i.e 3.73 and 3.77 respectively. Peroxide value of all the five tested oils ranges from 0.20 – 0.25 m eq/kg oil. Sterculia oil showed lowest totox value (2.67) with higher oxidative stability followed by sunflower (3.04) and soybean (3.81) oil. Higher TOTOX values were revealed by groundnut (5.45) and mustard oil (5.09) as shown in table-13.

Iodine value of the oils is directly proportional to the degree of unsaturation of the product which indicates the oxidative stability of the oil and was determined by following the method of **(Soares, S. and Rocha, F.R., 2018).**From the table-13 it can be concluded that the range of iodine value of Sterculia oil( 132-144) is much better compared to other oils present in the test which indicates th melting point and oxidative stability that are related to the degree of unsaturation. Iodine value also provides the estimation of these quality factors and greater the iodine value, more is the unsaturation and the higher the susceptibility to oxidation.

**Table-13**

**Iodine value and oxidation status of five seed oils namely Sterculia, Sunflower, Ground nut, Soybean, Mustard**.

| SEED OIL | Para-Anisidine Value (p-AV) | Peroxide value (PV)(meq./kg oil) | TOTOX (2PV + p-AV) | Iodine Value |
|---|---|---|---|---|
| Sterculia | 3.73 ± 0.64 | 0.023 ± 0.0034 | 2.67 ± 0.72 | 132-144 |
| Sunflower | 2.99 ± 0.45 | 0.025 ± 0.0052 | 3.04 ± 0.46 | 122-140 |
| Ground nut | 5.45 ± 0.68 | 0.018 ± 0.0004 | 5.48 ± 0.68 | 87-106 |
| Mustard | 5.09 ± 0.87 | 0.021 ± 0.005 | 5.13 ± 0.88 | 94-111 |
| Soybean | 3.77 ± 0.90 | 0.020 ± 0.0004 | 3.81 ± 0.90 | 120-134 |

*The values are mean ± SD of three independent experiments*

**G. Antioxidant assay :**

Using solvent extraction is frequently used for isolation of antioxidants and both the yield of extraction and antioxidant activity of extracts strongly depends on the solvent due to the different antioxidant potentials of compounds with different polarity. The total antioxidant capacity and polyphenol content vary greatly from one kind of oilseeds to another. Antioxidant activity and phenolic content of both edible portions and oilseeds of sterculia, sunflower, mustard, soybean and groundnut are measured by the use of DPPH, ABTS and NO assays which were carried out on these oilseeds in the laboratory. As we find that low IC50 value is inversely related to high antioxidant capacity of the extract then it can be denoted that IC50 is the concentration required to result in a 50% antioxidant activity i.e a 50% DPPH free radical scavenging or 50% reducing power compared with control. A smaller IC50 always presumably denotes higher antioxidant activity. **(Soong, Y.Y. and Barlow, P.J., 2004)**.

**DPPH radical scavenging activity** :

DPPH radical scavenging activity of all the five oils expressed in IC 50 (μg/ml) with BHT as standard as shown in the Fig:8. Soybean along with groundnut followed the highest antioxidant activities with lowest IC50 values .They are respectively 797.98 μg/ml and 815.19 μg/ml and after that comes Sterculia with a value of 825.96 μg/ml . Least DPPH radical scavenging activity with IC50 value of 1858.89 μg/ml and 1557.63 μg/ml respectively shown by mustard and sunflower oil. In sterculia , groundnut and soybean oil , there was no significant differences where as sunflower and mustard showed significant differences in DPPH activity.

**ABTS radical scavenging activity :**

ABTS radical scavenging activity of all the five oils represented in the fig:8. Highest ABTS radical scavenging activity with lowest IC50 of 166.70 was showed by groundnut followed by mustard , sterculia and soybean oil showing 198.51 μg/ml,225.27 μg/ml and 235.15 μg/ml respectively. Sunflower oil exhibited least antioxidant activity with highest IC50 of 17.28 μg/ml. No significant differences were observed in ABTS activity of groundnut, mustard , sterculia and soybean except sunflower.

**NO radical scavenging activity :**

NO radical scavenging activity of all the five oils expressed in IC50 (μg/ml ) is given as shown in the fig:8. Sterculia oil exhibited the highest NO radical scavenging activity with low IC50 value compared to other oils followed by mustard , groundnut , soybean and sunflower. The values are 114.98 μg/ml for Sterculia ;121.97 μg/ml,123.14 μg/ml, 230.24 μg/ml and 266.64 μg/ml for mustard ,groundnut , soybean and sunflower respectively. NO radical scavenging activity did not show any significant differences in all the five tested oils.

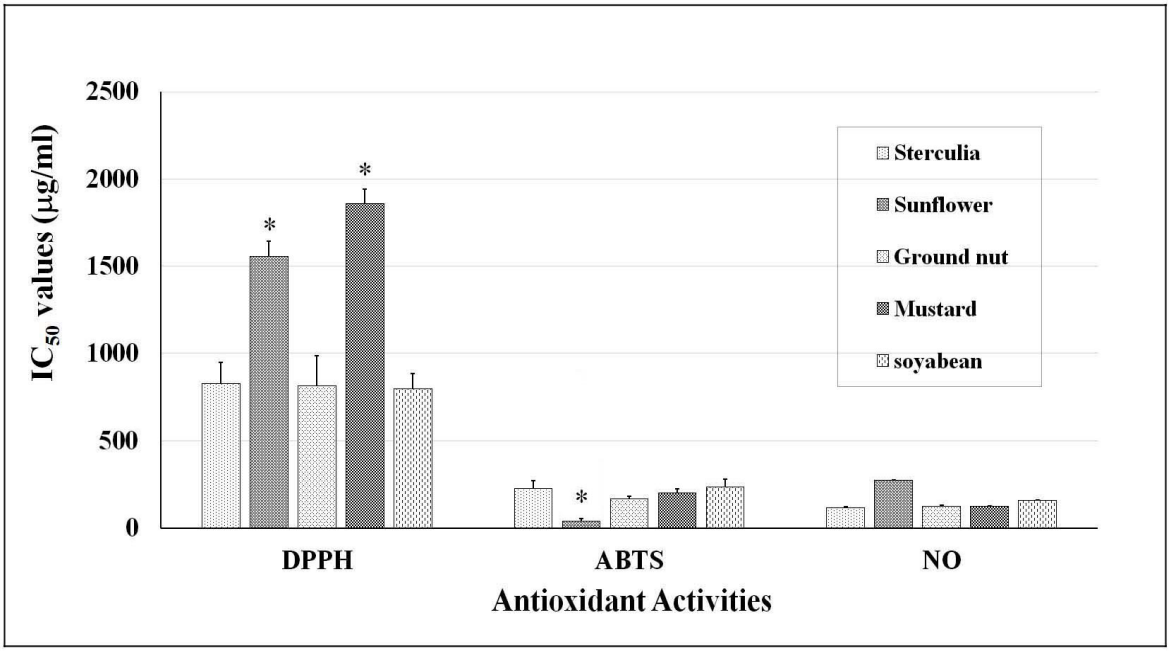



**Fig:-8. Antioxidant assay**

**H). CYTOTOXICITY ASSAY** :

In the cytotoxicity assay, the degree of acute and lethal toxicity obtained was indirectly related to the concentration of the oil samples examined. This lethality assay is primarily aimed at screening the seed oils for its cytotoxicity potential. **(Atolani *et al*.,2012)**

**a. Cytotoxicity assay of Sterculia oil-**

The promoting effect was increased by the increase in oil concentration where it was 10 , 20 ,40 and 80 µg/ml respectively. This indicates that the seeds of Sterculia can be used for the production of good quality of edible oil . It emphasizes for the production of edible oil extracted from Sterculia foetida seeds that can also be used in cosmetic and soap production. (**Vital *et al*., 2010) .**

From the fig-9, the result of cytotoxic activity of sterculia seed oil on both normal (MCF-10A and HB-2) and cancerous cell lines (BT-474 and AU-565 ) including the control with DMSO on MCF-10A cell line. Performing the MTT assay it showed clearly that the sterculia oil did

not show any cytotoxicity on both normal and cancer cell lines. On cancerous cell lines namely BT-474 and AU-565 at 10 µg/ml concentration when the sterculia oil is applied it exhibited 92.91% and 90.07% survivability of cells respectively. At 20 µg/ml concentrations,92.38% and 85.12% survivability of cells were obtained, at 40 µg/ml concentration 89.47% and 80.64% and finally at 80 µg/ml concentration it showed 82.46% and 74.01% survivability of cells were found.

Similarly on normal cell lines namely MCF-10A and HB-2 sterculia oil at 10 µg/ml concentrations showed 94.40% and 92.08 % survivability of cells respectively. At 20 µg/ml concentration it showed generally 92.62% and 85.35% respectively. Finally at 40 µg/ml and 80 µg/ml concentration it showed 86.61, 84.42% and also 80.56% and 76.89% survivability of cells. Depending on concentration it has been noted that there is a slight reduction in survivability of cells and this is the result of the effects of DMSO as evident by the control experiment with DMSO on MCF-10A cell lines.

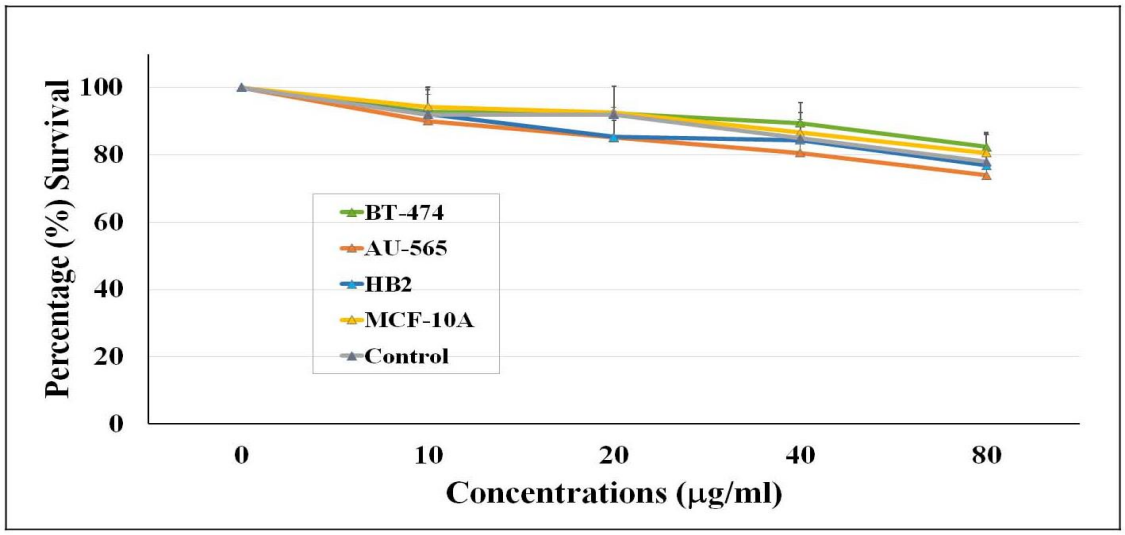



**Fig:-9. Cytotoxicity Assay of Sterculia oil.**

**b. Cytotoxicity assay of Bromosterculic acid-**

As bromosterculic acid possesses strong antimicrobial activity as compared to sterculia oil and other edible vegetable oils, so there was the need to study the cytotoxic activity of bromosterculic acid. Figure-10(a)and(b), shows the cytotoxic activity of bromosterculic acid on non-cancerous MCF-10A and cancerous cell lines MDA-MB-468. From the observation it is clear that the above extract is relatively toxic to the cancerous cells MDA-MB-468 whereas the decline in cell viability in the non-cancerous MCF-10A is due to the cytotoxic effect of DMSO and not bromosterculic acid. On cancerous cell the effect of bromosterculic acid is observed at a low dose of 5 µl with a LD50 dose around 10 µl whereas in non-cancerous cell lines MCF-10A, bromosterculic acid does not show any cytotoxicity rather the effect of DMSO on the cells**.**

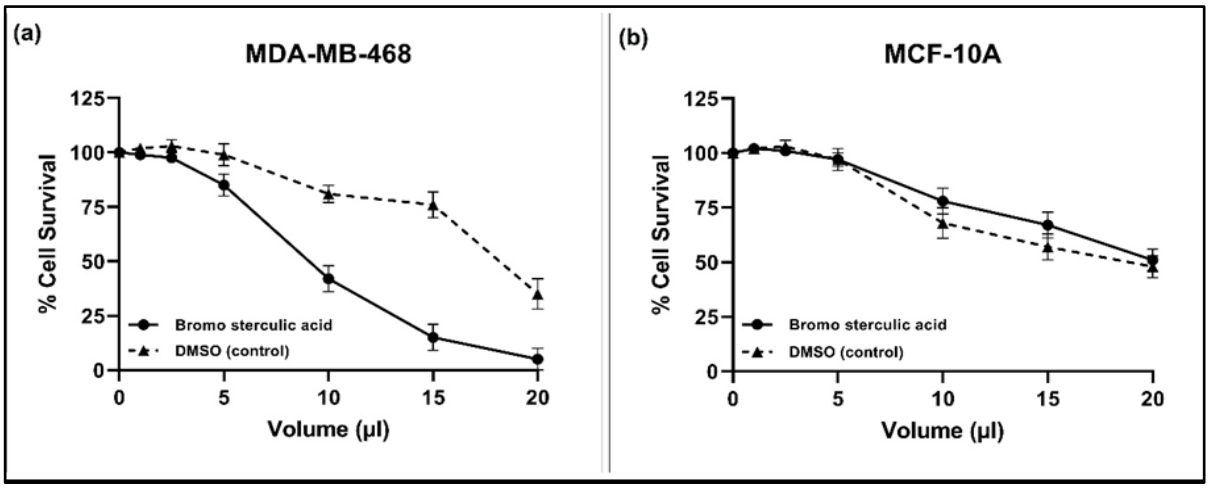



**Fig:10. Cytotoxicity assay of Bromosterculic acid against normal(MCF-10A) and cancerous (MDA-MB-468) cell lines.**

**Table-14 Statistical analysis using R-software to show the activity of bromosterculic acid on normal and cancer cell line.**

| Effect of bromosterculic acid on normal(No) and cancer(Ca) cell lines | Null and Alternative Hypothesis | p-value | Decision at 5% level |
|---|---|---|---|
| **5 $\mu l$** | $H_0: No = \text{Ca}$ against $H_1: \text{No} > \text{Ca}$ | 0.001 | $\boldsymbol{H_0}$ rejected |
| **10 $\mu l$** | $H_0: No = \text{Ca}$ against $H_1: \text{No} > \text{Ca}$ | 0.000 | $\boldsymbol{H_0}$ rejected |
| **15 $\mu l$** | $H_0: No = \text{Ca}$ against $H_1: \text{No} > \text{Ca}$ | 0.000 | $\boldsymbol{H_0}$ rejected |
| **20 $\mu l$** | $H_0: No = \text{Ca}$ against $H_1: \text{No} > \text{Ca}$ | 0.000 | $\boldsymbol{H_0}$ rejected |

In the above table, we have used four different concentrations to study the efficacy of Bromo-sterculic acid upon the normal and cancerous cell line. In this connection, we observe that the number of cell deaths is significantly higher in the case of cancerous cell lines compared to the normal cell line.The conclusion about the significant changes is made by the standard statistical testing procedure. Here we perform the student t-test with the 5% accuracy level for each of the concentration in between the normal and cancerous cell line. The p-value (in table-14) generated in each of the cases elucidates the efficiency of Bromo-sterculic acid that shows it is more stronger upon the cancerous cell than the normal cell-line.

## I). Molecular docking visualization-

Molecular docking of bromosterculic acid (ligand) with Bax and MDM2 proteins was separately performed by AutoDock 4.2, and the results were visualized by Chimera 1.9 and LigPlot+. Bromosterculic acid binds to Bax with the best conformation that has a minimum free binding energy of -11.4kcal/mole. It makes strong pi-sigma interaction with PHE-93, pi-alkyl and alkyl interaction with TRP-139, ARG-89 and PHE-92 as shown in fig:11(b) .The best

conformation of bromosterculic acid that binds to MDM2 has −11.6 kcal/mol binding energy. It makes strong hydrogen bond interaction with GLN-59 and pi-alkyl interaction with PHE-55. as shown in fig:11(a) and (b)

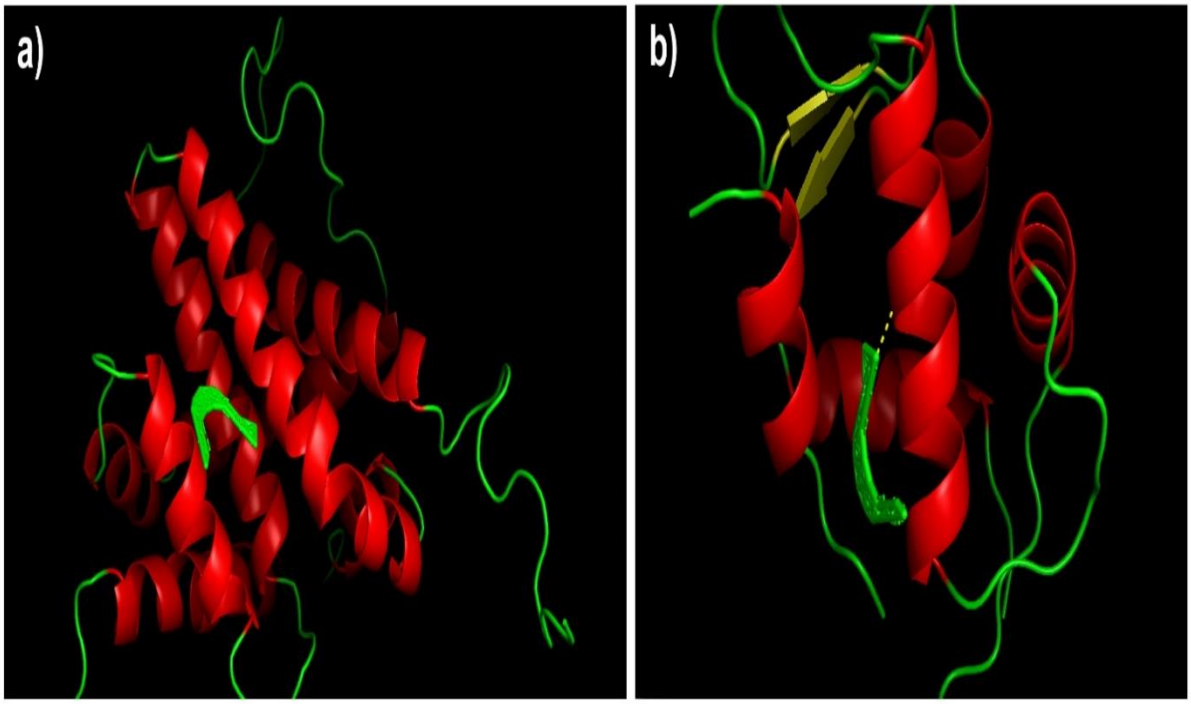
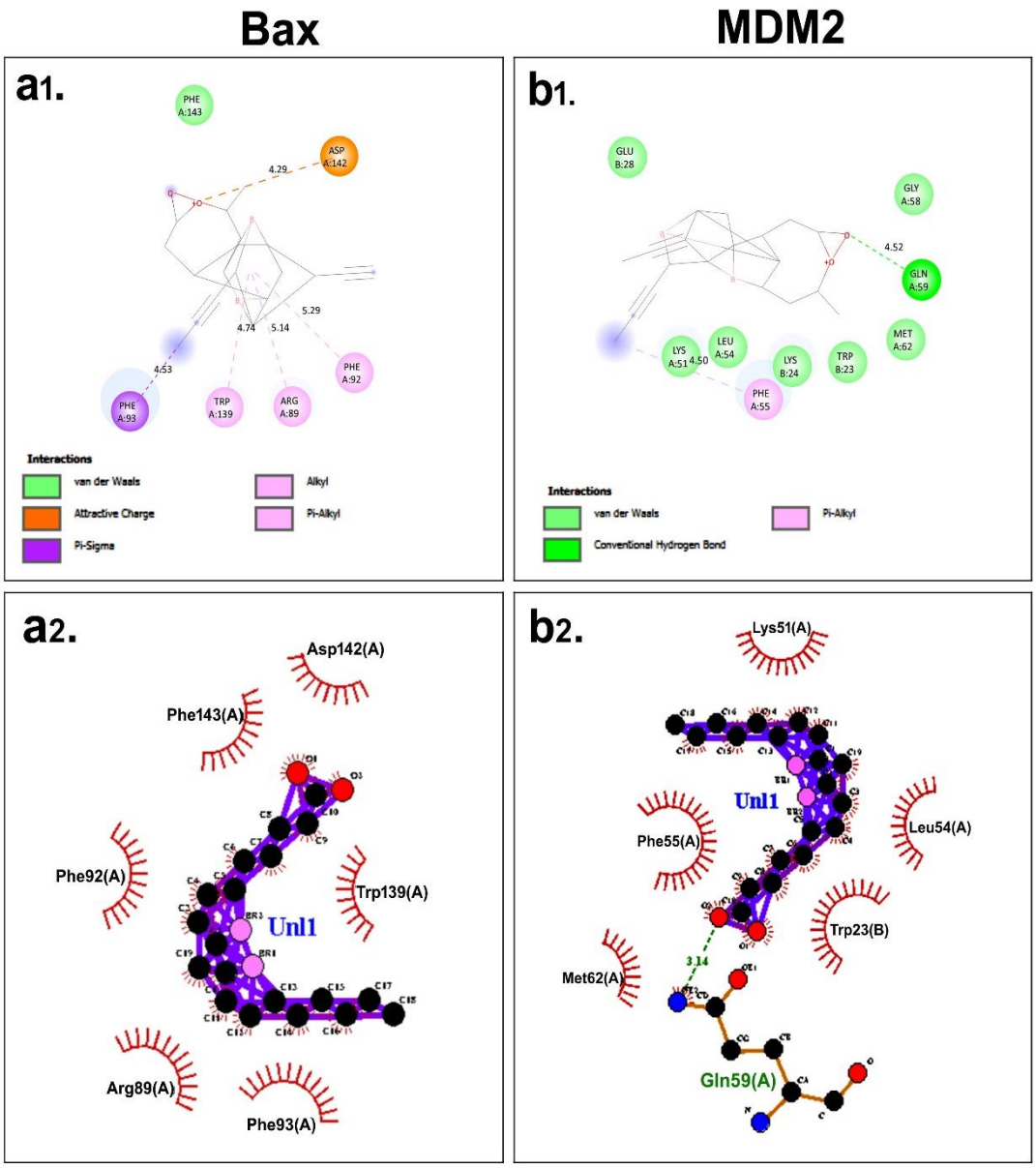



**Fig-11: protein-Ligand interactions between bromosterculic acid and Bax/MDM2 along with the 2D interaction plots within their active sites as displayed by Chimera 1.9. (a) Bromosterculic-BAX complex (b) Bromosterculic-MDM2 complex .**




**12(a1,a2) BAX** **12(b1,b2) MDM2**

**Fig:12 Hydrogen bonds and hydrophobic interactions formed between bromosterculic acid ligand and Bax (a) and between bromosterculic acid ligand and MDM2 (b) as analysed by LigPlot+.Bromosterculic acid is labelled as Unl1.**

**Drug-likeness and Toxicity Prediction-**

The major compound that is formed by brominating with bromo sterculic acid (9,10-dibromooctadecanoic acid) was subjected to drug-likeness and toxicity prediction using Lipinski's rule of five **(Ismail et al.,2020).**Lipinski's rule of five which determines the consistency of orally active drugs states that a drug molecule generally comply with more than

one of the following five rules : molecular mass <500 Da, high liphophilicity (expressed as logP < than 5), <5 hydrogen bond donors, <10 hydrogen bond acceptors and molar refractivity between 40 and 130.

| Compound | Mol.wt.(Da) | Hydrogen bond Donor | Hydrogen bond Acceptor | LogP | Molar refractivity | Rules satisfied |
|---|---|---|---|---|---|---|
| **Bromo-sterculic acid** | 442.27 | 1 | 2 | 4.62 | 106.15 | 5/5 |

The best protein-ompound configurations were chosen from the AutoDock 4.2 scoring function and it was placed according to their binding affinities, Discovery Studio Visualizer 4.1 client, Chimera 1.14 and using LigPlot **(Wallace et al., 1995)** used for post-docking analyses as shown in the fig-7.

## J). Anti-microbial activities:

The antimicrobial activity of Bromosterculic acid and Java olive oil (Sterculia oil) extracted from the seeds are compared against four other edible oils (Sunflower, Mustard, Soybean and Groundnut) present in the market against various bacterial and fungal strains to prove its effectiveness.

**Fungicidal Activity of bromosterculic acid against Sterculia oil and other edible oils-**

The tested solution which showed Inhibition Zone Diameter (IZD) greater than 11 mm were regarded as favourable antimicrobial activity **(Bayer et al., 1966)**. Fig.10 shows the antifungal activity of bromosterculic acid against *Penicillium chrysogenum* and *Aspergillus niger* along with sterculia oil and other four edible vegetable oils (namely groundnut, soybean, sunflower and mustard). As compared to sterculia oil and other edible vegetable oils, new synthetic bromosterculic acid is much more active on two pathogenic fungal species.

In case of *Penicillium chrysogenum,* bromosterculic acid showed antifungal activity with IZD value of 14.56, 17.53 and 21.87mm at a concentration of 0.25, 0.50 and 1mg/ml respectively. Inhibition activity is directly proportional to the concentration. At 1mg/ml concentration sterculia oil revealed IZD value of 18.2mm, mustard oil 10.06 mm, sunflower oil 17.13 mm, soybean oil 12.63mm and groundnut oil 12.2mm against *Penicillium chrysogenum.*

Bromosterculic acid also showed good antifungal activity against *Aspergillus niger* with IZD value 13.73, 17.93 and 21.26 mm respectively at a concentration of 0.25, 0.5 and 1 mg/ml, whereas sterculia oil showed IZD value of 15.06 mm at 1mg/ml concentration. Fungicidal activity of other edible oils showed IZD value in the range of 8mm to 17 mm at different concentrations.

MIC value of sterculia oil and bromosterculic acid against both the fungal species was 0.06 mg/ml and 0.015 mg/ml respectively.

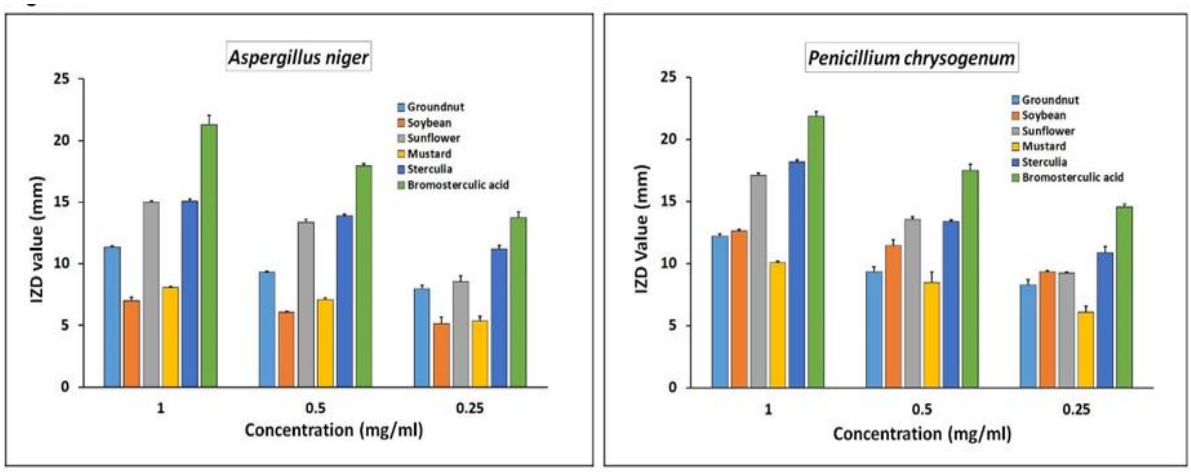



**Fig:13 Fungicidal activity of bromosterculic acid against Sterculia oil and other edible oils.**

**Bactericidal Activity of bromosterculic acid against Sterculia oil and other edible oils-**

Here in figure-11 shows the bactericidal activity of bromosterculic acid against *Bacillus subtilis* and *Xanthomonas* sp along with sterculia oil and other four edible vegetable oils (namely ground nut, soybean, sunflower, and mustard). As compared to sterculia oil and other edible

vegetable oils, the new synthetic bromosterculic acid is much more active on two pathogenic bacterial species. In case of *Bacillus subtilis*, bromosterculic acid showed antibacterial activity with IZD value of 14.36, 15.46 and 20.2 mm at a concentration of 0.25, 0.50 and 1 mg/ml respectively.Inhibitory activity increases with concentration. At 1mg/ml concentration, sterculia oil revealed IZD value of 14.13 mm, mustard 12.93 mm, sunflower 16.03mm, soybean 13.13mm and groundnut 13.56 mm against *Bacillus subtilis.* Here Ampicillin and DMSO has been used as positive and negative control respectively.

Bromosterculic acid also showed good antibacterial activity against *Xanthomonas sp* with IZD value of 16.26, 17.6 and 21.43 mm at a concentration of 0.25, 0.50 and 1.0 mg.ml respectively whereas sterculia oil showed IZD value of 13.36mm in 1.0 mg/ml concentration. Bactericidal activity of other edible oil showed 1ZD value ranging from 10mm to 16 mm at different concentration.

In case of *Xanthomonas sp*, bromosterculic acid and sterculia oil showed MIC value of 0.007mg/ml and 0.06 mg/ml respectively. Bromosterculic acid and sterculia oil revealed MIC value of 0.015 mg/ml and 0.06 mg/ml respectively against *Bacillus subtilis.*

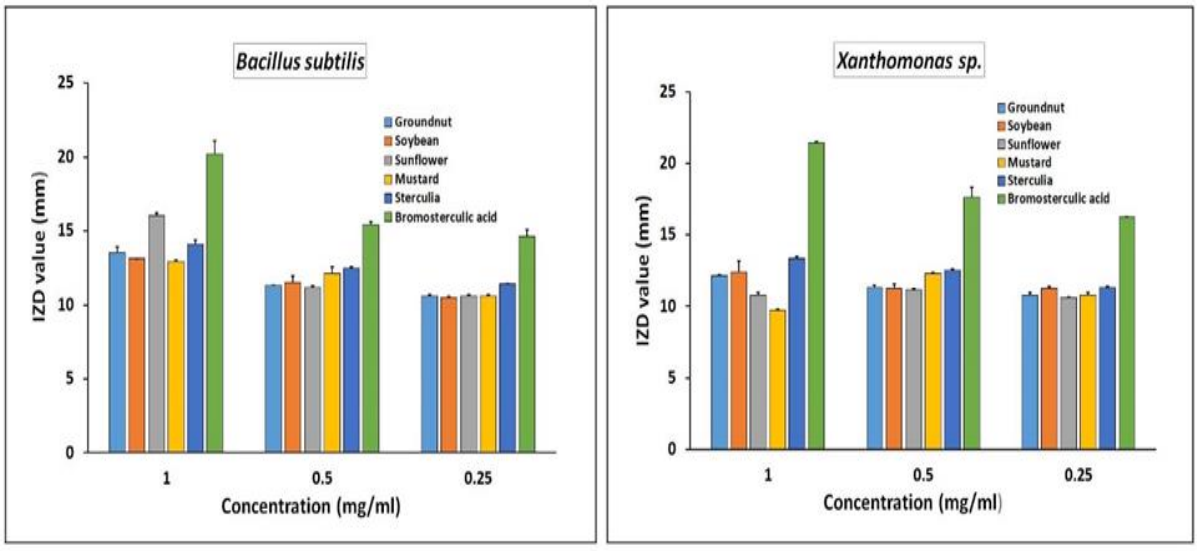



**Fig:14. Bactericidal activity of bromosterculic acid against Sterculia oil and other edible oils.**

**Table 15: Comparison of bactericidal inhibition activity between Bromosterculic acid and all other oils in case of *Xanthomonas sp***

| Levels | Null and Alternative Hypothesis | p-value | Decision at 5% level |
|---|---|---|---|
| **Bromosterculic acid(Br) vs Groundnut(Gn)** | $H_0: Br = \text{Gn}$ against $H_1: \text{Br} > \text{Gn}$ | 0.02 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Soybean(Soy)** | $H_0: Br = \text{Soy}$ against $H_1: \text{Br} > \text{Soy}$ | 0.02 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Sunflower(Sun)** | $H_0: Br = \text{Sun}$ against $H_1: \text{Br} > \text{Sun}$ | 0.02 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Mustard(Mu)** | $H_0: Br = \text{Mu}$ against $H_1: \text{Br} > \text{Mu}$ | 0.01 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Sterculia(St)** | $H_0: Br = \text{St}$ against $H_1: \text{Br} > \text{St}$ | 0.02 | $\boldsymbol{H_0}$ rejected |

**Table 16: Comparison of bactericidal growth inhibition activity between Bromosterculic acid and all other oils in case of *Bacillus subtilis.***

| Levels | Null and Alternative Hypothesis | p-value | Decision at 5% level |
|---|---|---|---|
| **Bromosterculic acid(Br) vs Groundnut(Gn)** | $H_0: Br = \text{Gn}$ against $H_1: \text{Br} > \text{Gn}$ | 0.04 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Soybean(Soy)** | $H_0: Br = \text{Soy}$ against $H_1: \text{Br} > \text{Soy}$ | 0.04 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Sunflower(Sun)** | $H_0: Br = \text{Sun}$ against $H_1: \text{Br} > \text{Sun}$ | 0.08 | $\boldsymbol{H_0}$ accepted |
| **Bromosterculic acid(Br) vs Mustard(Mu)** | $H_0: Br = \text{Mu}$ against $H_1: \text{Br} > \text{Mu}$ | 0.04 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Sterculia(St)** | $H_0: Br = \text{St}$ against $H_1: \text{Br} > \text{St}$ | 0.06 | $\boldsymbol{H_0}$ accepted |

**Table 17: Comparison of fungicidal growth inhibition activity between Bromosterculic acid and all other oils in case *Penicillium chrysogenum*.**

| Levels | Null and Alternative Hypothesis | p-value | Decision at 5% level |
|---|---|---|---|
| **Bromosterculic acid(Br) vs Groundnut(Gn)** | $H_0: Br = \text{Gn}$ against $H_1: \text{Br} > \text{Gn}$ | 0.02 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Soybean(Soy)** | $H_0: Br = \text{Soy}$ against $H_1: \text{Br} > \text{Soy}$ | 0.03 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Sunflower(Sun)** | $H_0: Br = \text{Sun}$ against $H_1: \text{Br} > \text{Sun}$ | 0.1 | $\boldsymbol{H_0}$ accepted |
| **Bromosterculic acid(Br) vs Mustard(Mu)** | $H_0: Br = \text{Mu}$ against $H_1: \text{Br} > \text{Mu}$ | 0.01 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Sterculia(St)** | $H_0: Br = \text{St}$ against $H_1: \text{Br} > \text{St}$ | 0.1 | $\boldsymbol{H_0}$ accepted |

**Table 18: Comparison of fungicidal growth inhibition activity between Bromosterculic acid and all other oils in case of *Aspergillus niger*.**

| Levels | Null and Alternative Hypothesis | p-value | Decision at 5% level |
|---|---|---|---|
| **Bromosterculic acid(Br) vs Groundnut(Gn)** | $H_0: Br = \text{Gn}$ against $H_1: \text{Br} > \text{Gn}$ | 0.02 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Soybean(Soy)** | $H_0: Br = \text{Soy}$ against $H_1: \text{Br} > \text{Soy}$ | 0.01 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Sunflower(Sun)** | $H_0: Br = \text{Sun}$ against $H_1: \text{Br} > \text{Sun}$ | 0.07 | $\boldsymbol{H_0}$ accepted |
| **Bromosterculic acid(Br) vs Mustard(Mu)** | $H_0: Br = \text{Mu}$ against $H_1: \text{Br} > \text{Mu}$ | 0.01 | $\boldsymbol{H_0}$ rejected |
| **Bromosterculic acid(Br) vs Sterculia(St)** | $H_0: Br = \text{St}$ against $H_1: \text{Br} > \text{St}$ | 0.09 | $\boldsymbol{H_0}$ accepted |

# Chapter-5

# Discussions

Oilseed crops are the second most important factor of agricultural economy, that comes next only to cereals within the segment of field crops. Though the country has achieved much self-sufficiency in foodgrains production it had produced surplus amounts of rice and wheat with mounting food stocks.But in cases of oilseeds and pulses our country is facing a serious shortages. Being the fourth largest edible oil economy in the world, India contributes about 10 percent of the world oilseeds production.6-7% of the global production of the vegetable oil and nearly 7% of the protein meal, India is one of the largest importers of edible oils in the world. India imported over 11 million tonnes of edible oils during 2012-13 that accounts for more than half of the total consumption in the country. Oilseeds sector consists of an important position in the Indian agricultural sector, that covers an area of 26.5 million hectares (14.8% of gross cropped area) and total production of over 29 million tonnes in triennium during 2011-12 (**GOI,2012**). Oilseed industry accounts for about 10 percent of the total value of output from agriculture. Two major factors or policy initiatives that have significantly impacted the development of Indian oilseeds sector.The first policy is the setting up of " Technology Mission on Oilseeds" in 1986 that gave a major boost to the Government in increasing production of oilseeds and this resulted in an impressive increase in the production of oilseeds from about 11 million tonnes in mid 1980's to 21.5 million tonnes in 1993-94 (**Jha *et al*.,2012**). The important aspect that had a significant impact on edible oilseeds/ oil industry is the policy of liberalization and globalization in the early 1990's that allowed the free import of edible vegetable oils and reduction in import tariffs. This policy has given rise to a significant increase in the imports of edible oils and thus had some adverse impact on domestic production. However from the last

decade the production of oilseeds showed an upward trend, that went up from about 25 million tonnes in early 2000's to about 32.5 million tonnes in 2010-2011.This has been a record production. As per May 15, 2014 during the 3rd advance estimates by Ministry of Agriculture the production of 9 major oilseeds is about 32.4 million tonnes during 2013-14.As production of edible oilseeds has increased during the last decade, but due to rise in total consumption there has also been a share of imports, as in total consumption has also increased from about 33 percent in 2005-06 to about 53.5 percent in 2012-13 **(ICAR 2013,2013a,2013b,2013c).**This happened primarily due to certain factors such as increase of income of majority households, changing food habits etc. Now as there is an increasing demand for edible oils and to reduce the country's dependence on import of edible oil, there is an immediate need to increase edible oilseeds production in the country. There has been a long competing demand for agricultural land from various crops cultivated in India and thus the chances for increasing the area under oilseeds has been very limited. Thus the production of oilseeds can only be increased only if productivity is improved significantly and farmers get their remunerative and attractive prices, better market access ,technology and other infrastructure facilities.The most difficult hurdle that the oilseeds farmers face has been that most of oilseeds are grown under rainfed conditions and only 28 percent of the area under oilseeds are irrigated.

Important changes observed in the Indian agriculture over the last three decades shows that there has been a change in the cropping pattern. Significant changes involves the shift of plot from coarse cereals to rice, wheat and commercial crops, mainly fruits and vegetables and crop intensification. The share of cereals in the GCA declined from about 59.6 percent in TE 1983-84 to about 51.7 percent in TE 2010-11.This changes in the amount of area under oilseeds that became more pronounced after the mid-80's owing to concerted efforts of the government. However oilseeds plot declined in the second-half of 1990's because of drought and falling edible oil prices due to cheap imports of palm oil from Malaysia and Indonesia. Variability in

the yield of oilseeds has shown to be a major factor for production variability during all decades, that results of an indication of high yield risks associated with oilseeds. This is generally because yield appears to have been the primary source of growth in output of most edible oilseeds in the last decade. However the current yields of major edible oilseeds are much lower than the average production in the world and potential yields. Firstly, Soybean comes at a top position both in terms of area and production as its share in output **(Kajale, J. and Shroff, S., 2013 )** is over 40 percent which is followed by rapeseed-mustard **(Swain, Mrutyunjay 2013)** being the second important crop with estimated share of 24.5 percent of oilseeds output during 2010-11.Groundnut being the third which was the preponderant crop during the period of 1980s-1990 **(Swain, M., 2013)**, it lost its share and accounted for only 23.7 percent total production.

Soybean has shown growth both in the area and production that has been un-parallel during the last four decades **(Masuda, T. and Goldsmith, P.D., 2009 ).** Area for cultivation has increased from 0.04 million ha in 1971-73 to about 9.8 million ha during 2011-12, while production increased from about 30 thousand tonnes to 11.6 million tonnes during that period. Along with this production of soybean also increased from 691 kg/ha in 1971-73 to 1186 kg/ha in 2011-2012. In relation to area expansion growth of soybean crop has witnessed a phenomenal increase that had contributed to about 80 percent to increased production.

Rapeseed-mustard also an important oilseed crop that occupies the second position after Soybean. Area for cultivation of rapeseed-mustard increased from 3.46 million hectare in 1971-1973 to 4.03 million hectare in 1981-83 and further to 6.34 million hectare in 1991-93.The growth rate of rapeseed- mustard was nominal during the 2000's and later part of twenty first century compared to its negative growth rate (-1.78%) during the nineties. Among institutional constraints non-availability of quality inputs and services are the most important aspect. Further insufficient knowledge of importance of modern technology and management and use of new

instruments by the farmers lack inadequate knowledge about farming to reduce the effect of disease and pest management and poor extension services were significant constraints that are faced by the farmers.

The groundnut acreage in the country showed a significant rise in the 1980's while during the nineties there was a significant plunge. The negative dive in growth in area under groundnut continued in the next decade and recorded statistically negative growth. Thus groundnut production became stagnant in the last three decades. Cultivation of groundnut is less profitable and more risky when compared with other competing crops and thus leads to the decline in area and production of groundnut in the country. Irregular yields and uneven growth of groundnut in our country led to a serious problem of groundnut production.

Sunflower has also witnessed a wide undulation both in area and production in the country **(Singha *et al*., 2014 ).** Between 1991 to 1996 the output of sunflower oil was relatively stable at an average of 1.2 million tonnes and acreage at 2.2 million tonnes. During the next five years between 1996-2001 crop area declined to 1.5 million hectare and production fell to 0.8 million tonnes. Again during 2003-2007 the production of sunflower recovered but plunged in 2008-09 and reached the lowest level 0.52 million tonnes in 2011-2012. Post-harvest management and marketing related constraints were perceived as the most important constraints in sunflower cultivation. Exploitation by market intermediaries along with lack of processing facilities and poor marketing system and lack of access to markets followed by low and fluctuating prices, shortage of human labour and low profitability of sunflower compared with competing crops.

Now as discussed above the edible oils constitute an important part of food habits in every Indian households and accounts about 6.7% of food expenditure. Demand for edible oils in the country increased at a steady rate at an annual compound growth rate of about 5.5% during the

last decade which is mainly attributable to rising income levels and living standards and changing food habits **(Rao, M.N., 2014).** In terms of volumes palm oil, soybean oil and mustard oil are the three largest consumed edible oils in India but demand for edible oils is expected to grow in the future at much higher rate and there has been a significant gap between demand and supply of edible oils because of slow growth in domestic oilseeds production **(Sharma, H.O. and Rathi, D., 2013)** and shifting of plot to other high value crops **(Roy, R., 2013).** This gap was filled up through imports that accounted for about 57% of the total oil consumption. Given the current scenario and demographic fundamentals, the edible oils have a favourable demand growth outlook over the medium to long-term **( Sharma, V.P., 2017).**

Because of the need of edible oil in the global market, this *Sterculia foetida* tree could be an alternative source of edible oil in Asian and African countries , and rich natural sources of oil that can be cultivated in every un-utilized lands or even in waste lands with very nominal water and nutrient supply which is very pertinent to developing countries. It shows very high growth rate and have very long (more than 100 years) life span. This tree produces a very large number of seeds i.e. 450-2080 kg dry seeds/tree/year. Seed kernel of *Sterculia foetida* produces about 68% oil which is very similar to other edible oils in their chemical properties, which could allow it to be easily substituted with other edible oils. Seeds of *S. foetida* yielded substantial amounts of oil (58.7 g per 100 g seed). The moisture content of Sterculia seeds was 5.28%, which is low as compared to other tested oils. The seeds contained significant amounts of crude oil, protein, lipid and minerals. Heavy metals were present in very low amount except zinc which is essential for proper functioning of the immune system. Among the alkaline earth metals, sodium , magnesium and potassium was found to be highest in seeds of Sterculia compared to other oilseeds.

FAME production rate are comparable with other seeds. Though biodiesel productivity in terms of FAME yield is higher in soybean but the overall oil production is much lower. Substantial

amount of oil and FAME are produced from Sterculia seeds. So biodiesel production from this resource is one of the effective ways to overcome the problems associated with energy crisis and environmental issues.

Fatty acids like palmitic acid, linoleic acid, linolenic acids, stearic acid and oleic acids were common in all the five oils. Results reveal that sterculia oil consists of highest amount of fatty acids, which includes the SFA, MUFA and PUFA. Highest percentage of polyunsaturated fatty acids such as linoleic acids and linolenic acids and monounsaturated fatty acids such as oleic acids along with sterculic acid (CFA) are present in sterculia oil that helps in raising the high density lipoprotein (HDL) i.e the good cholesterol which assists in the removal of triacyl glycerols from the bloodstream (J.Lunn, & Theobald, 2006).Moreover, the unique cyclopropenoid fatty acid i.e. sterculic acid [namely 8-(2-Octacyclopropen-1-yl) octanoic acid] was found in the Sterculia seed oil (Kale et al, 2011; Vipunngeun and Chanida, 2009). Sterculic acid is a potent natural product to fight against obesity by suppressing a bodily enzyme associated with insulin resistance, which could indirectly help with reducing belly fat (Bao et al, 2003). It is also known for the inhibition of SCD1 (Stearoyl-CoA desaturase-1), a major enzyme involved in the control of lipid metabolism and has emerged as a potential therapeutic target for reducing obesity and its associated metabolic complications including insulin resistance and hepatic steatosis (Ortinau, et al, 2013). This sterculic acid directly inhibits SCD activity, possibly by a turnover-dependent reaction, without affecting the processes required for adipocyte differentiation, SCD gene expression or SCD protein translation (Gomez et al, 2003). So Sterculia oil has a promise to act to reduce some factors causing obesity. Lowest TOTOX value and higher iodine value of Sterculia oil indicates its higher oxidative stability and presence of greater number of double bonds in the fatty acid moieties which further supports that this oil would be beneficial for edible purposes.

No significant differences in IC50 values among the oils were observed as measured by NO radical scavenging activity. ABTS radical scavenging activity showed significant difference only with sunflower oil whereas DPPH showed differences with sunflower and mustard oil. Sterculia oil showed lower IC50 values in all the antioxidant activity assays. So Sterculia oil is comparable to other vegetable oil based on their radical scavenging activity.

Furthermore, this oil did not reveal any cytotoxic effect even at 40µg/ml concentration against normal (MCF-10A and HB-2) and cancerous cell lines (BT-474 and AU-565). Similarly bromosterculic acid showed relatively toxicity to the cancerous cells (MDA-MB-468) whereas the decline in cell viability in the non-cancerous (MCF-10A) is due to the cytotoxic effect of DMSO and not the acid. On cancerous cell the effect of bromosterculic acid is observed at a low dose of 5 µl with an LD50 dose of around 10 µl whereas in non-cancerous cell lines MCF-10A, bromosterculic acid does not show any cytotoxicity rather the effect of DMSO on the cells. So, this oil has no toxicity on human beings and can be assumed to be safe for human consumption.

In-silico apoptotic ability of bromosterculic acid with Bax/MDM2 was also studied. The protein-ligand interactions of bromosterculic acid with Bax/MDM2 that have been obtained by docking are represented. LigPlot+ (**Wallace et al., 1995**) has been used to determine the intermolecular bonds involved. In apoptosis the activation of Bax induces mitochondrial membrane permeabilization leading to the release of cytochrome-c which causes cancerous cells to die **(Garrido et al., 2006).** Lots of recent research focuses on Bax activators that can serve as a promising direct target for cancer therapy that has the potential of overcoming chemotherapy and radioresistance. Similarly targeting MDM2 can be also helpful in combating breast cancer **(Qin et al., 2015).** Here, bromosterculic acid showed interactions with various MDM2 amino acids residue. This also suggests that bromosterculic acid could activate P-53 by inhibiting formation of P-53-MDM2 complex thereby letting the P-53 perform its normal

tumor suppressing activity. Also, bromosterculic acid did not violate Lipinski's rules, suggesting the drug-likeness of bromosterculic acid that could be suitable for oral administration.

Bromo-sterculic acid showed much greater fungicidal activity compared to sterculia oil and other edible vegetable oils in this study. When applied at 1000 ppm (1mg/ml) concentration, bromosterculic acid showed antifungal activity with inhibition zone determination (IZD) value of (21.87 mm) compared to sterculia oil (18.2 mm), mustard oil (10.06 mm), sunflower oil (17.13 mm), soybean oil (12.63 mm) and groundnut oil (12.2 mm) respectively against *Penicillium chrysogenum* . As previously reported essential oil obtained from the peel of Citrus grandis (Tao and Liu, 2012) showed antimicrobial activity against *Penicillium chrysogenum* with an inhibition zone of (20.71 mm) which is lesser compared to the bromo-sterculic acid. In case of *Aspergillus niger*, Bromosterculic acid showed good antifungal activity with IZD value of (17.93 mm) compared to sterculia oil (13.86 mm) and other edible oils used in this study showed IZD value in the range of (7-13 mm) when applied at 500 ppm (0.5 mg/ml) concentration. But previous studies reports that barks of *Sterculia villosa* has shown mild activity against *Aspergillus niger* with IZD value of (8-9 mm) when applied at 500 ppm (0.5 mg/ml) concentration **(Haque et al., 2014).**

Bromosterculic acid also showed good anti-bacterial activity compared to sterculia oil and other edible vegetable oils present in the study. At 0.5 mg/ml concentration it showed good inhibition activity with IZD value of (15.46 mm) compared to sterculia oil (12.5 mm) , mustard oil (12.13 mm), sunflower (11.16 mm), soybean (11.53 mm) and groundnut (11.3 mm) against *Bacillus subtillis*. Previously reported that extracts from the bark of *Sterculia villosa* have shown activity against *Bacillus subtilis* with IZD value of (8 mm) applied at 500 ppm (0.5 mg/ml) which is much lower compared to sterculia oil and the new derivative bromosterculic acid. Similarly against *Xanthomonas* sp., bromosterculic acid showed good antibacterial

activity with IZD value of (21.43 mm) compared to Sterculia oil (13.36 mm) at 1 mg/ml concentration. Other edible oils used in the study showed IZD value in the range of (9-12 mm). As reported in previous studies essential oil obtained from Oriental melon (*Cucumis melo* L.) have shown antibacterial activity against *Xanthomonas* sp. with IZD value of (15.05 mm) which shows far less activity compared to bromosterculic acid **(Bajpai et al., 2010).** This is generally due to the combination of bromine and sterculic acid which together acts as a good antibacterial agent. Thus from this study it can be defined that efficiency of plant extracts and their effective compounds as antimicrobial agents can be used to restrict the growth of food-borne pathogenic bacteria and also it can be used as antifungal agent in food protection from mycological and mycotoxicological contamination **(Kocić-Tanackov et al., 2017).** MIC value of both sterculia oil and bromosterculic acid revealed that both of them showed zero inhibition activity at a certain concentration against both bacterial and fungal strains respectively.

All the parameters of sterculia oil recommends that the seed oil of S. foetida, L. may be an alternative source of safe edible oil. Also the seeds of *Sterculia apetala* are commonly used in some tropical areas in Mexico for human and animal nutrition **(Herrera-Meza et al, 2014).** Consumption of *S. apetala* seed oil in Zucker rats reduces anxiety-like behaviour and some behavioural alterations in locomotor activity tests **(Herrera-Meza et al, 2017).**

Because of the need of edible oil in the global market, seeds of this tree would be a viable resource of nutritious, non-toxic edible vegetable oil. This plant has a wide range of distribution in all around the world so it is not invasive nor eco-destructive. It can be cultivated in un-utilized lands or even in waste lands with very nominal water and nutrient supply which is very relevant to developing countries.This tree is useful for backyard planting; Boundary marker; Coastal protection and stabilization, commercial planting; erosion control, large roadside tree, riparian management, shade tree, specimen tree; urban greening, wild grafting **(Orwa *et al.* 2009).** This oil could also be potential sources for future energy supply. The seed oil of

*Sterculia foetida* would be alternative rich natural sources of edible oil for the global market without exhausting the agricultural lands and also holds promise for meeting global demand for vegetable oils and for enhancing nutritional, industrial, and biofuel properties of vegetable oils. Along with this the main aim of this natural synthetic compound is targeting the tumor vasculatures which is essential for tumor treatment and to offer a secure platform for the targeted delivery of anti-cancer drugs for the treatment of cancer. This will in turn help reduce the cytotoxic side effects of anti-cancer drugs on normal cells **(Olusanya et al., 2018).** Moreover, the most important finding of this study can be attributed using bromosterculic acid as antimicrobial and anticancer agent as natural products from medicinal plants that represent a fertile ground for the development of novel anticancer agents **(Wang et al., 2012)** which can be used for the formation of future medicines for the treatment of infectious diseases with targeted drug delivery for pharmaceutical industry. Selective drug targeting is the need of the current therapeutic regimens for increased activity on cancer cells and reduced toxicity to normal cells **(Khan & Gurav, 2018).**

Statistically, also it has been proven using R software as shown in the study that the efficiency rate of bromosterculic acid is much higher in controlling the growth of pathogenic microorganisms as compared to the sterculia oil. Thus this plant can be attributed as a potential substitute for drugs that are being used today, such as ampicillin which has been found to show multidrug-resistant bacteria **(Gopinath et al., 2015).**

# Chapter-6

# Conclusion

Study suggests that all over the world there is a huge scarcity of edible vegetable oil and the demand for it universally increasing day by day. But apart from the edible vegetable oils present in the market (groundnut, mustard, soybean, sunflower, rice bran oil, olive oil) there are no other optional sources. So there is an urgent need to find a suitable alternative source of vegetable oil from under-utilized oilseeds that can help to meet the global scarcity of vegetable oil market demand**.** This new synthetic product, bromo-sterculic acid [8-(1, 2-dibromo-2-octylcyclopropyl) octanoic acid] from sterculia seed oil which shows good antimicrobial and cytotoxic activity. Its target specific cytotoxic activity towards cancerous cells provide a greater opportunity to develop new drugs for cancer treatment with nominal side effects**.**

From the above research work performed it can be concluded that the seeds of sterculia compared to other oilseeds in this experiment produces about 63.4 gm of oil from 100 gm of seeds. The other oilseeds produces much less oil i.e Sunflower 47.5 gm, Groundnut 49.8 gm, Mustard 31.5 gm and Soybean 16.3 gm of oil produces the least of all respectively.

Lipid and protein content was also high in Sterculia compared to other oils. The amount of lipid content was 58.4 gm/100 gm and protein content was 38.43gm. High protein content in sterculia seeds is a good factor as it can be included in the everyday diet for a good source of natural vegetable protein as most percentage of population in Indian subcontinent suffer from protein deficiency.

Moisture retention capacity of the seeds of sterculia is the lowest about 5.28gm/100 gm compared to other edible oils in this study. Lower moisture uptake is ideally good so that these seeds can be stored for a longer period without any dampness or humidity can damage them.

Among the heavy metals, Sterculia seeds are found to contain lowest amount of copper (16.68 mg/kg), lead (12.7 mg/kg) and manganese (10.7 mg/kg). Iron (938.68 mg/kg) is present in high amount compared to other edible oils present in the market.

Among the alkaline earth metals, sodium (350.67 mg/kg), magnesium (3436 mg/kg) and potassium (19858 mg/kg) was found to be highest in seeds of Sterculia compared to other oilseeds.

Fatty acid composition of all the tested oils shows that the seed oil of Sterculia contains highest amount of total fatty acids that includes both PUFA and SFA and also CFA. As oxidation of oil depends on the totox value that is calculated by the formula AV+2PV to specify the overall oxidation condtion of the oil lower the totox value better is the quality of oil, so Sterculia oil showed lowest Totox value (2.67) with higher oxidative stability that is followed by sunflower (3.04) and Soybean oil (3.81). Higher totox value was revealed by groundnut (5.45) and mustard oil (5.09).

Radical scavenging activity of the oilseeds of sterculia, sunflower, mustard, groundnut and soybean. As it has been denoted that low IC50 value is inversely related to high antioxidant capacity of the extract then it can be denoted that IC50 is the concentration required to result in a 50% antioxidant activity. A smaller IC50 value always presumably denotes higher antioxidant activity.

Cytotoxicity assay performed on the sterculia oil shows that this oil doesn't have any toxicity on human health which has been revealed from MTT assay. Both the sterculia oil and bromosterculic acid have shown non toxicity towards normal cell line but showed good resistance against cancerous cell lines. As by previous reports of (**Petropoulos *et al.*,2021**) linseed oil, luffa oil and cucurbit oil showed a slight toxicity against the non-tumorous porcine liver primary culture (PLP2) cell line.While linseed oil was non-effective against cervical

carcinoma (HeLa) cell lines, but pumpkin oil, luffa oil and purslane oil showed some effectiveness. Moderate toxic effects were reported for solvent extracts of purslane plant oils (stem and leaves) against human lung (K562 and A549) and breast (MCF-7 and MDA-MB-435) cancer cell lines, colon cancer (HT-29) and cervical cancer (HeLa) **(Tian et al., 2013).** However to the best of our knowledge there have been no reports regarding the cytotoxic effects of bromosterculic acid against cancer cell lines and the results of our study could be helpful for the utilisation of various underutilized species .One of the most important part of cancer treatment is the specificity towards targeted cancer cells without showing any toxicity towards normal cells **(Sylla et al., 2012).**

Molecular docking study of bromosterculic acid with Bax exhibited strong pi-sigma interaction interaction with PHE-93, pi-alkyl and alkyl interaction with TRP-139, ARG-89 and PHE-92 whereas with MDM2 revealed strong hydrogen bond interaction with GLN-59 and pi-alkyl interaction with PHE-55.

Finally *Sterculia foetida* seed oil have a good antimicrobial property. It is found to inhibit maximum against particular strains of bacteria and fungi. Sterculic acid being a cyclopropenoid fatty acid present in the seed oils also has good antimicrobial property.

Thus based on all these tested parameters, it may be concluded that this oil can be used as an alternative viable source of safe edible oil in the market compared to other oils and also to be used as biofuel that would help in fulfilling the future energy demands and produce less toxic pollutant to keep the environment clean and healthy.

# Chapter-7

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